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(54) **COMPOSITIONS COMPRISING SIRNA AND PLASMIDS**

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(51) **Int. Cl.**  
**C07H 21/00** (2006.01)  
**C07H 21/02** (2006.01)

(52) **U.S. Cl.**  
USPC ..... **536/24.5**; 536/23.1

(58) **Field of Classification Search**  
None  
See application file for complete search history.

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(57) **ABSTRACT**

The present invention relates to the combinatorial use of an siRNA targeted against an endogenous gene to knock out or knock down expression of the endogenous gene in a host and a delivery of a polynucleotide encoding the gene in a delivery vehicle/expression vector to the host to provide expression in the host of the protein encoded by the polynucleotide.

**7 Claims, 42 Drawing Sheets**

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Amino Acid Sequence of Human eIF5A1

Hypusination site

51  
 pHM6 - eIF5A1 # 2 MADDLDFETG DAGASATFPM QCSALRKNKF VVLKGRPCKI VEMSTSKTGK  
 genbank MADDLDFETG DAGASATFPM QCSALRKNKF VVLKGRPCKI VEMSTSKTGK  
 Consensus MADDLDFETG DAGASATFPM QCSALRKNKF VVLKGRPCKI VEMSTSKTGK

acetylation site K47 K50



UQ

51  
 # HGHAKVHLVG IDIFTGKKYE DICPSTHND VPNIKRND FQ LIGIQDGYLS  
 genbank HGHAKVHLVG IDIFTGKKYE DICPSTHND VPNIKRND FQ LIGIQDGYLS  
 Consensus HGHAKVHLVG IDIFTGKKYE DICPSTHND VPNIKRND FQ LIGIQDGYLS

PKC K67

101  
 # LLQDSGEVRE DLRLPEGLD KEIEQKYDCG EEILITVLSA MTEEA AVAIK  
 genbank LLQDSGEVRE DLRLPEGLD KEIEQKYDCG EEILITVLSA MTEEA AVAIK  
 Consensus LLQDSGEVRE DLRLPEGLD KEIEQKYDCG EEILITVLSA MTEEA AVAIK

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# AMAK  
 genbank AMAK  
 Consensus AMAK

PKC = putative protein kinase C phosphorylation site

UQ = putative ubiquitination site (K67)

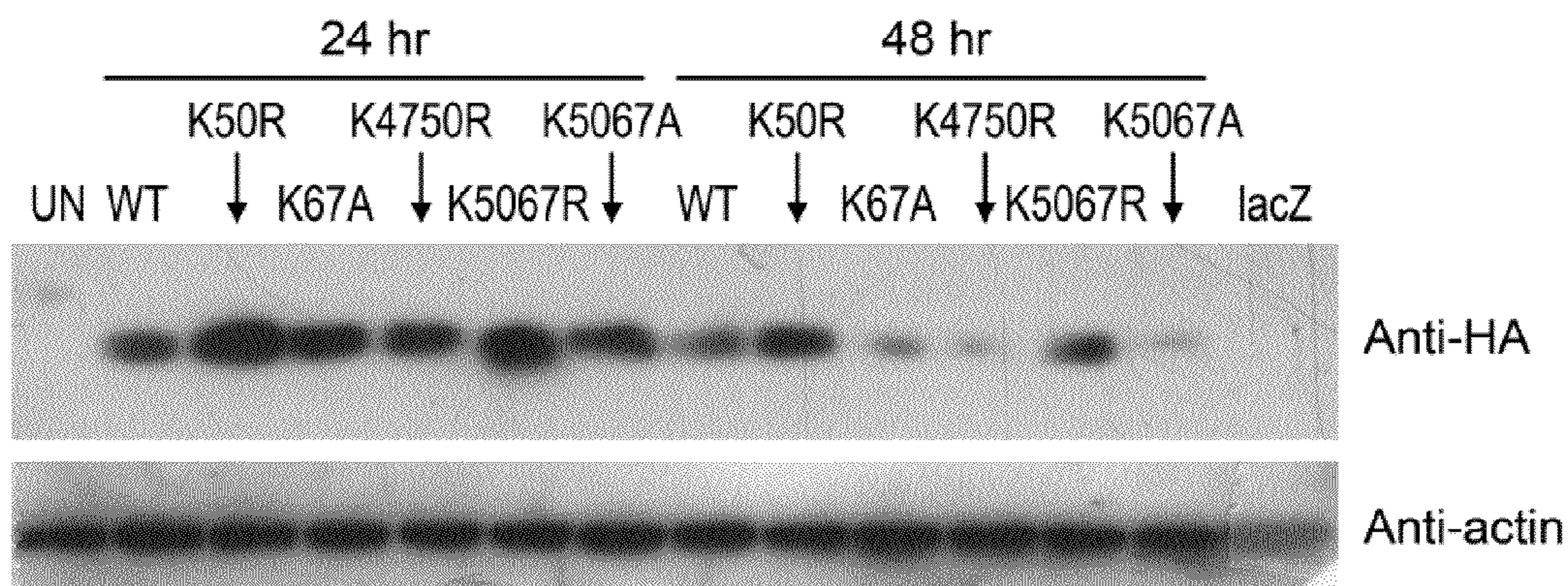
K47 = confirmed acetylation site

K50 = confirmed hypusination site

pHM6-eIF5A1 # 2 = predicted amino acid sequence of human eIF5A1 in pHM6 vector

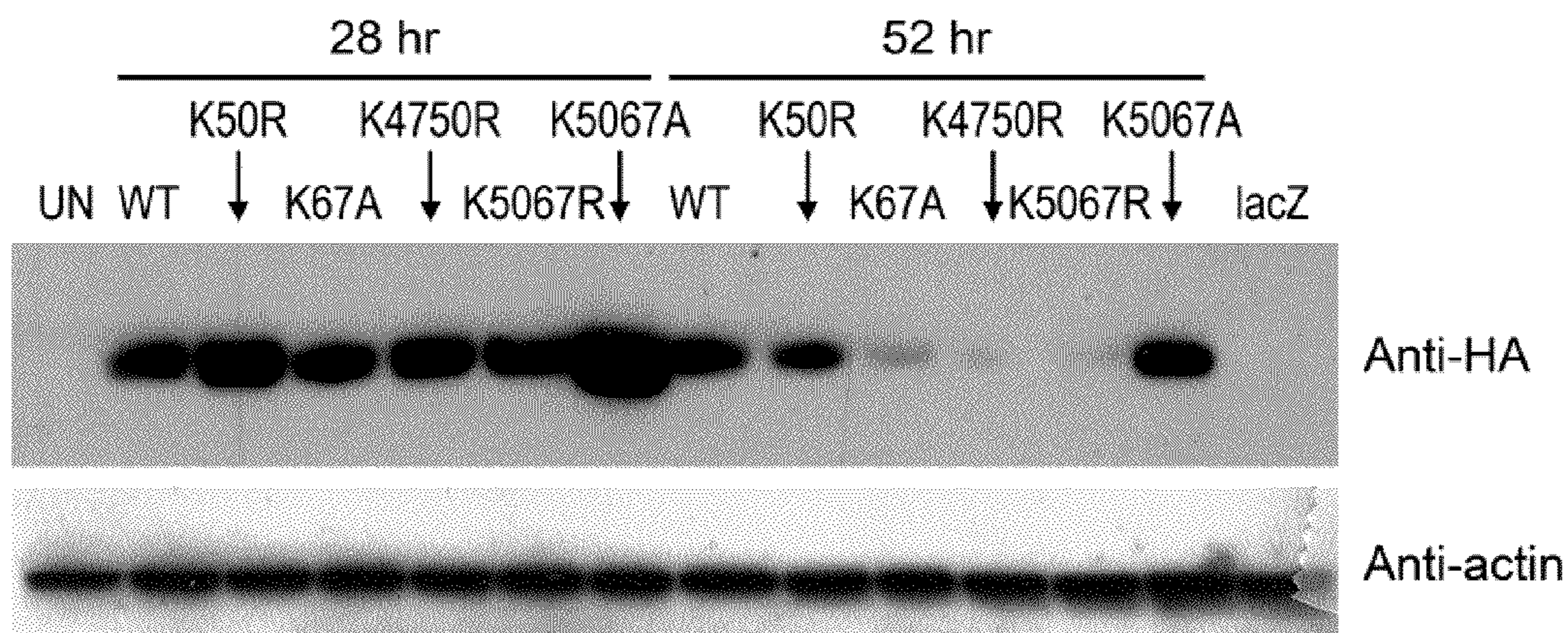
Genbank = amino acid sequence of human eIF5A1 (ACCESSION # NP\_001961)

FIG.1



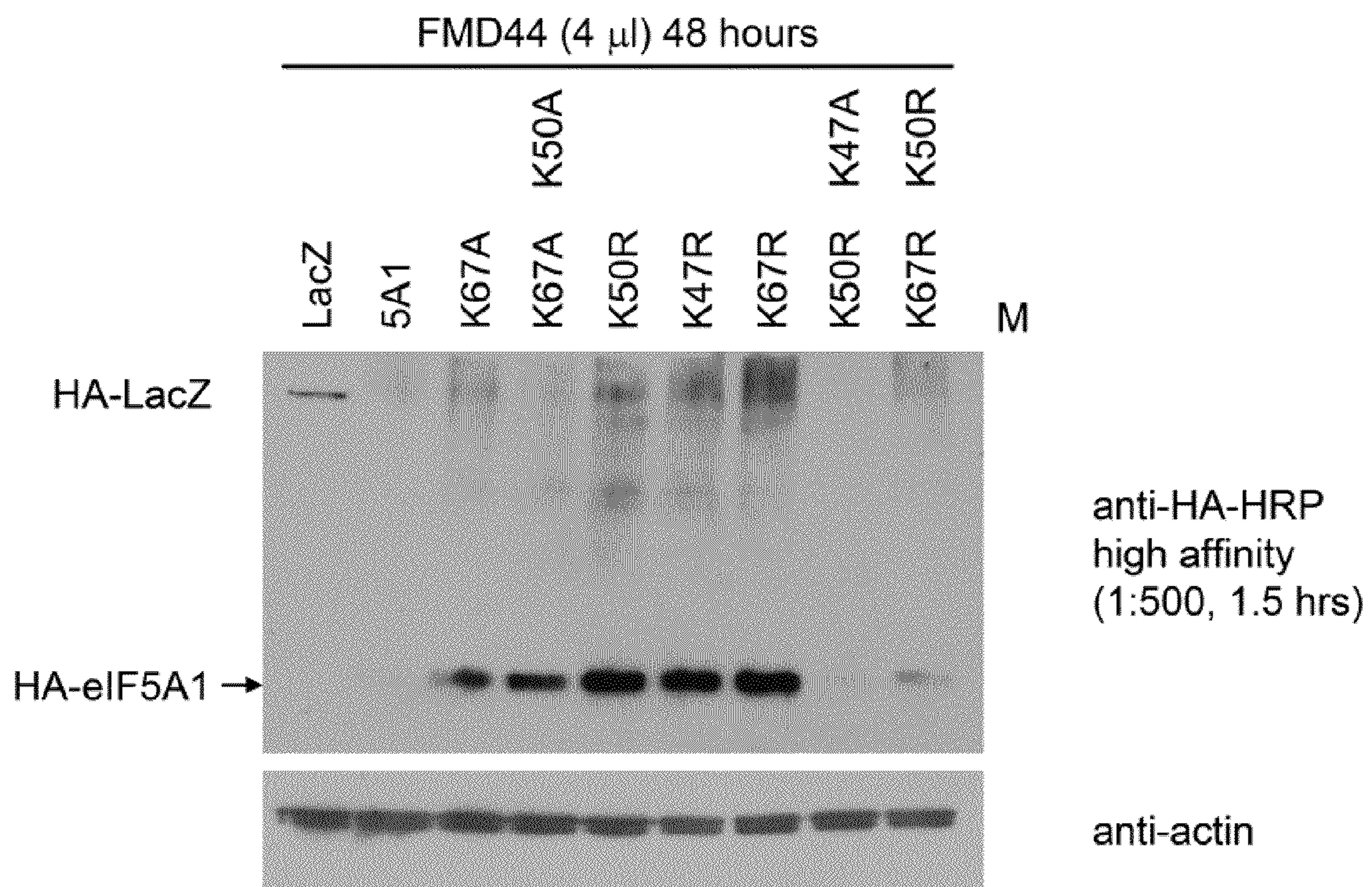
Mutation of eIF5A1 at K50 and K67 increases accumulation of transfected protein.

FIG.2A



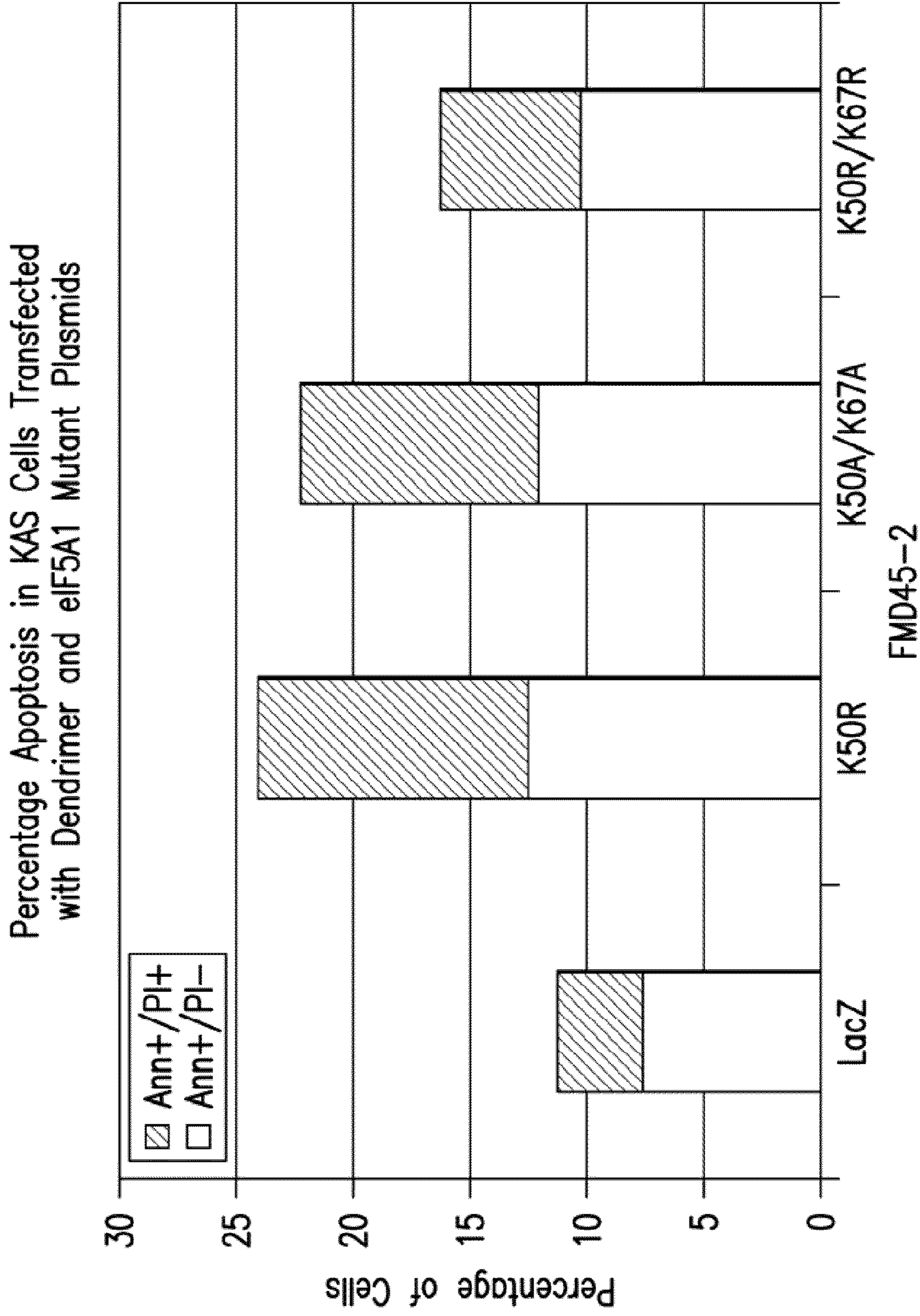
Mutation of eIF5A1 at K50 and K67 increases accumulation of transfected protein.

FIG.2B



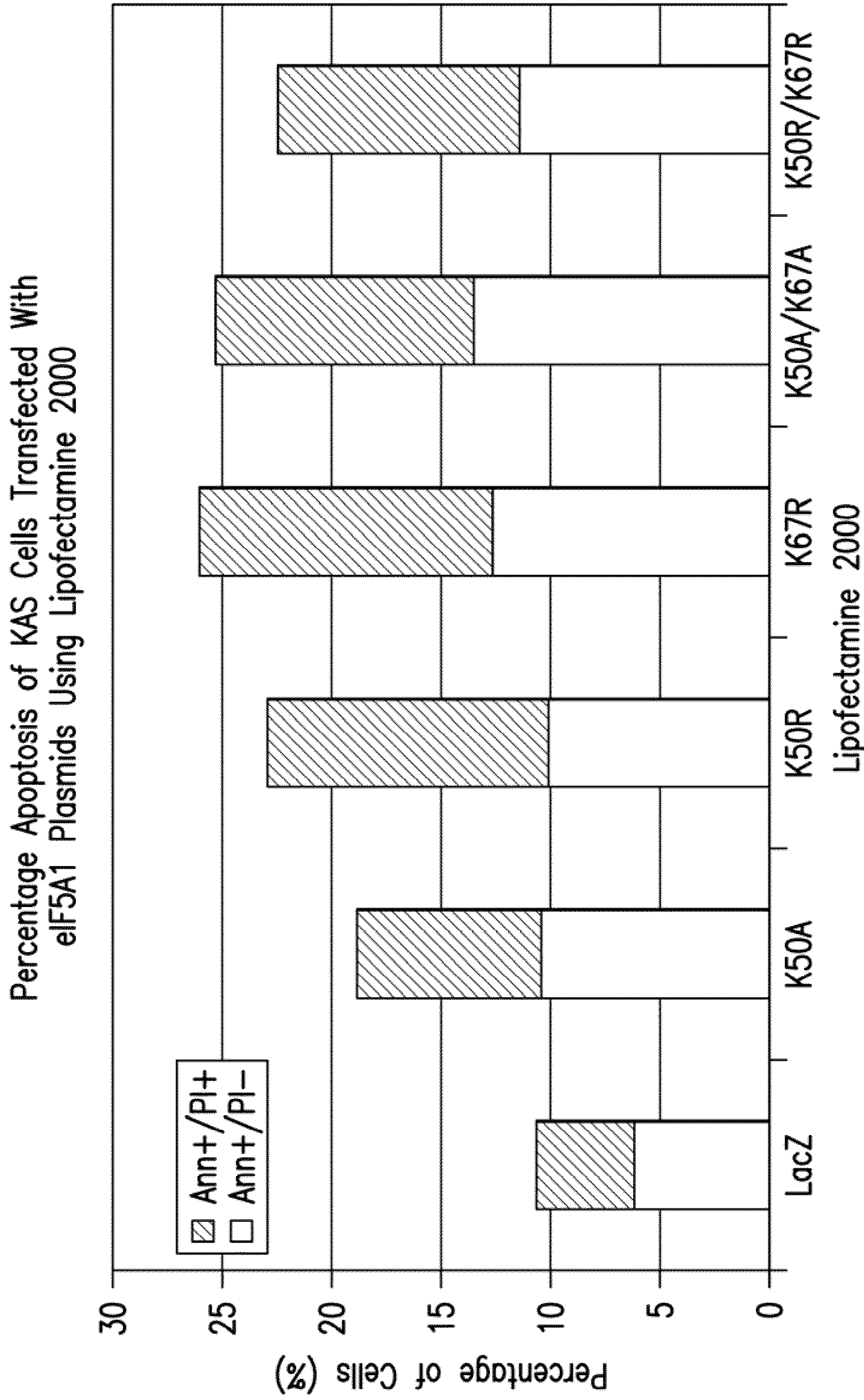
Mutation of eIF5A1 at K47, K50 and K67 increases accumulation of transfected protein.

FIG.3



Mutation of eIF5A1 at K50 and K67 result in induction of apoptosis when transferred to KAS cells.

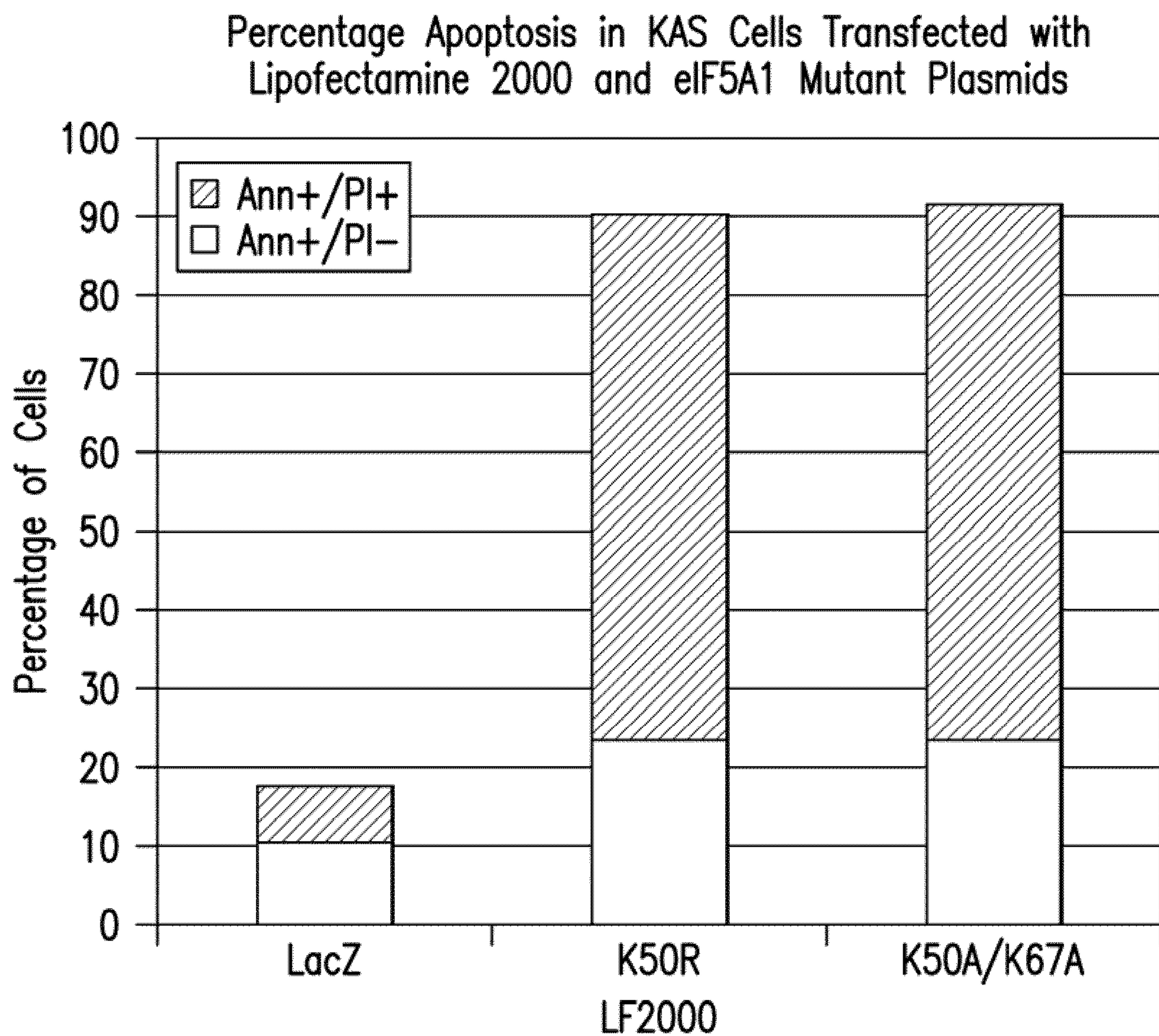
FIG.4



Mutation of eIF5A1 at K50 and K67 result in induction of apoptosis when transferred to KAS cells.

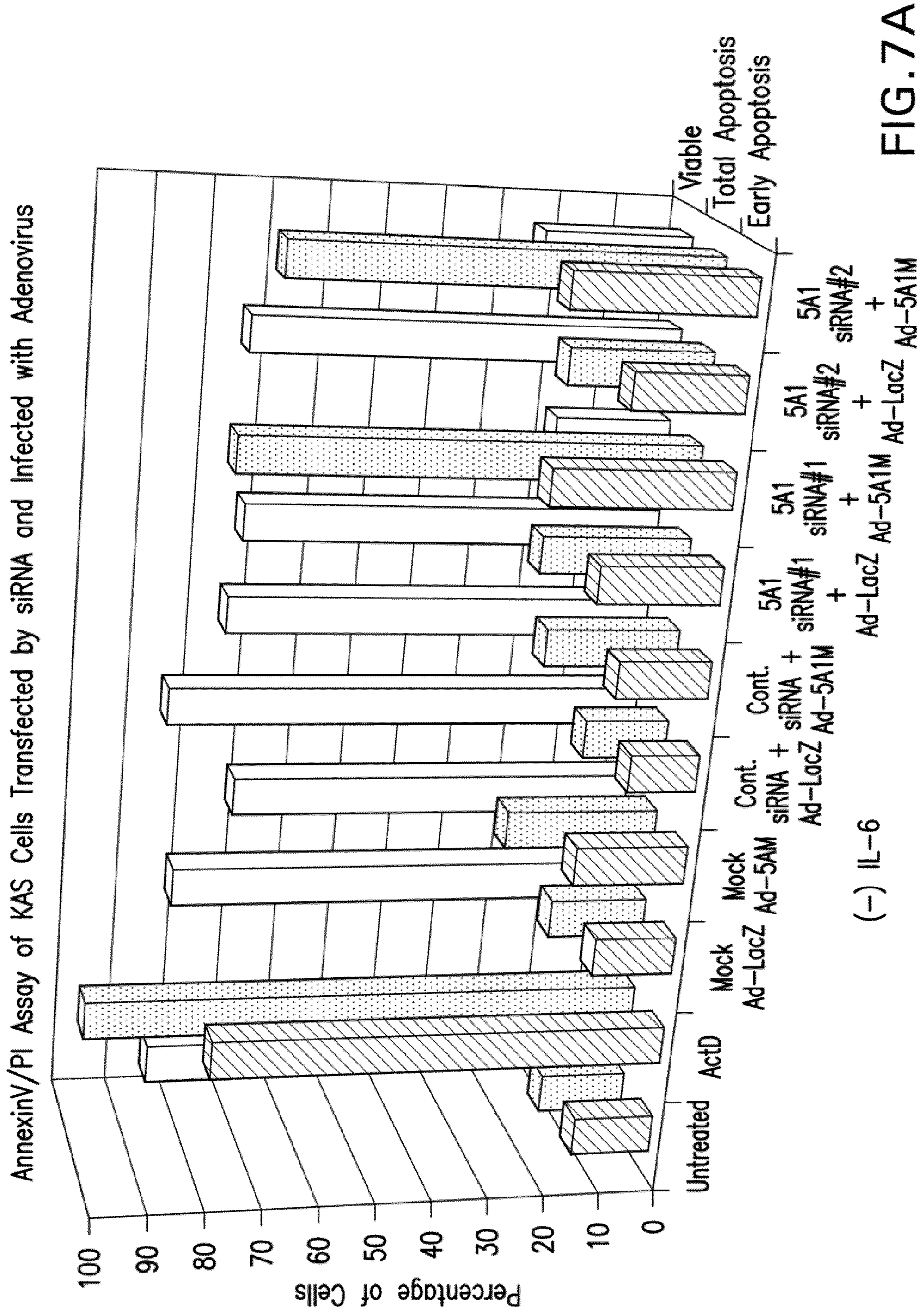
FIG.5

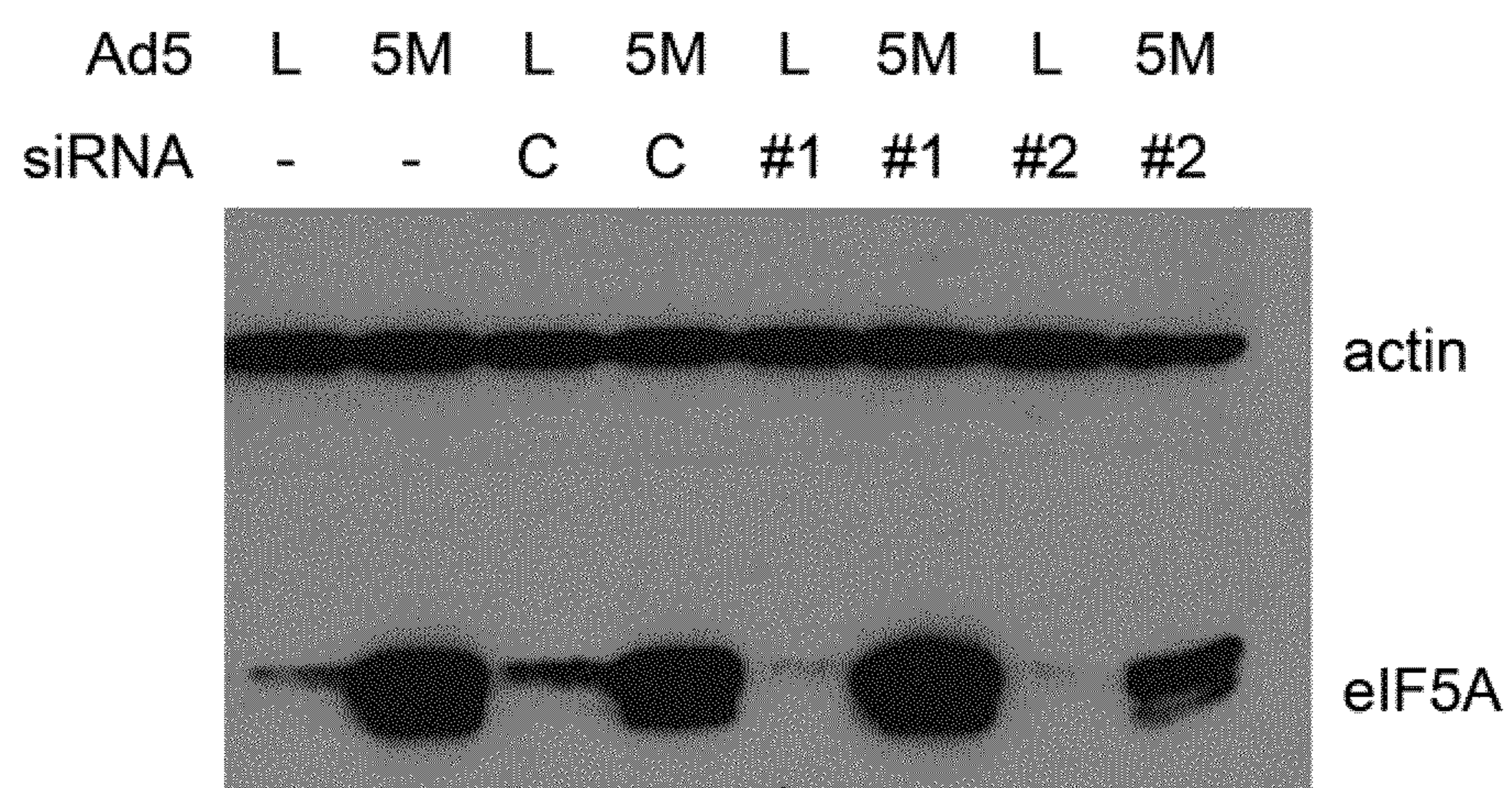




Mutation of eIF5A1 at K50 and K67 result in induction of apoptosis when transferred to KAS cells.

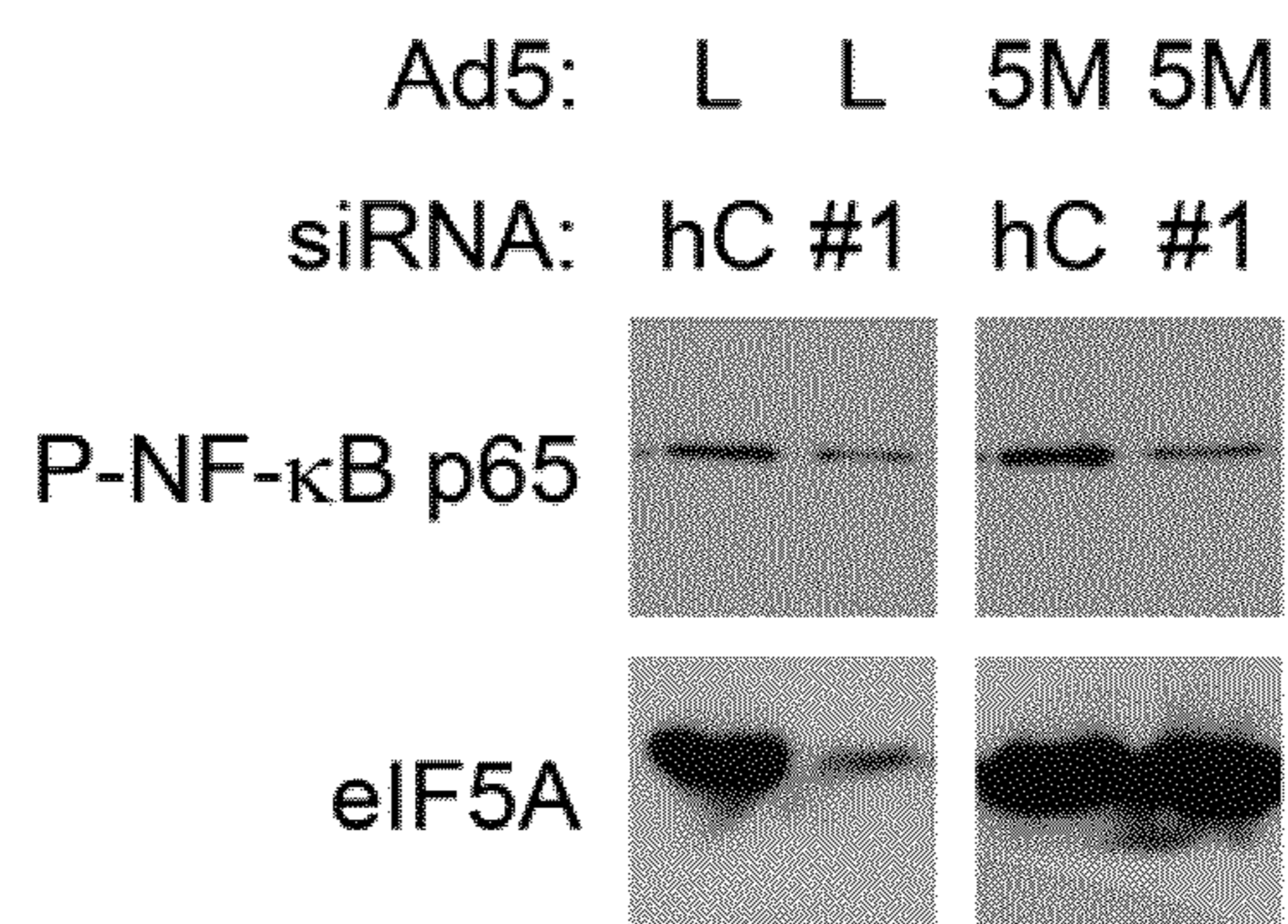
FIG. 6





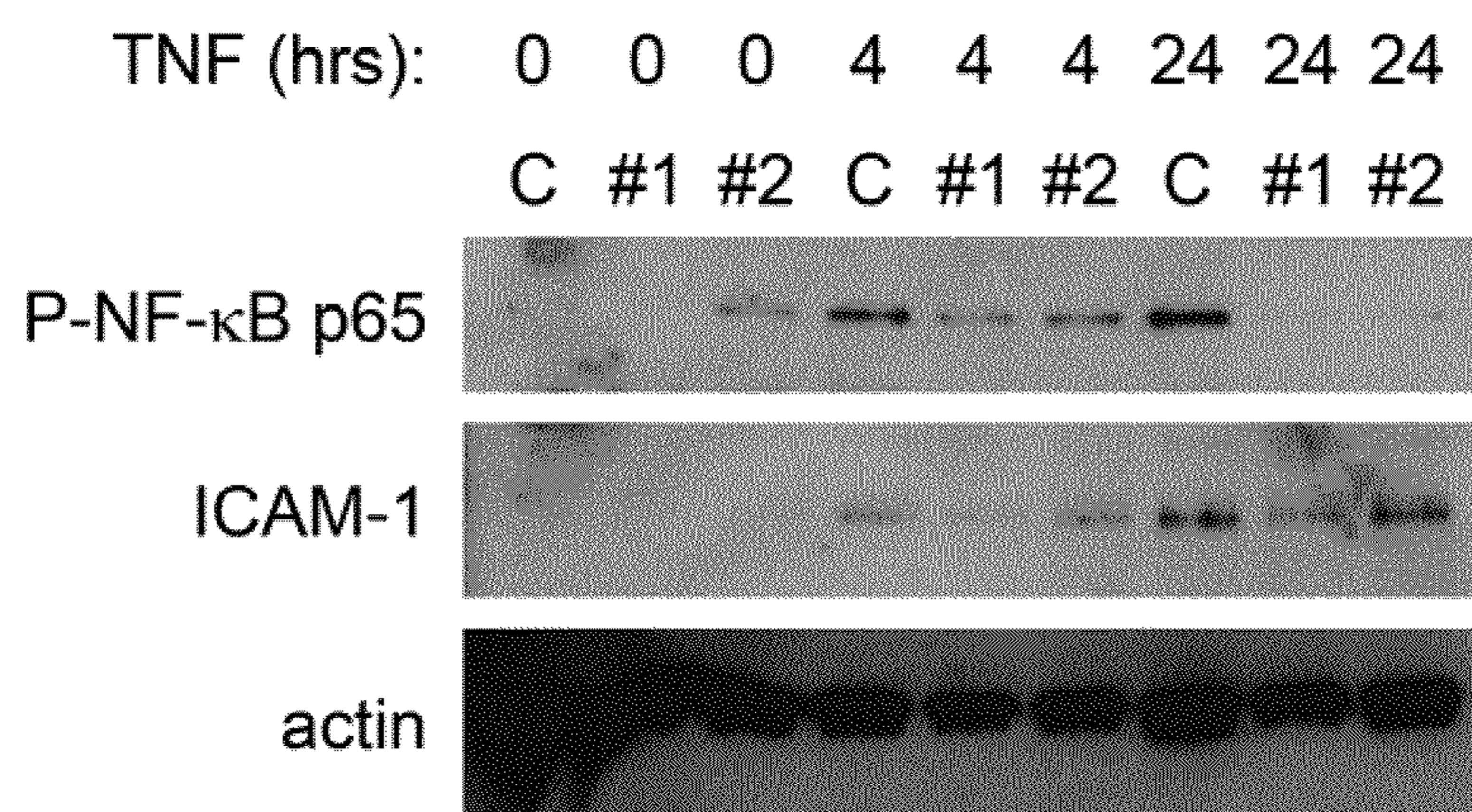
Pre-treatment with eIF5A1 siRNA #1 reduces expression of endogenous eIF5A1 but allows accumulation of RNAi-resistant eIF5A1<sup>k50A</sup> expressed by adenovirus

FIG.7B



Pre-treatment with eIF5A1 siRNA #1 prior to Adenovirus infection reduces expression of phosphorylated NF- $\kappa$ B in human multiple myeloma cells

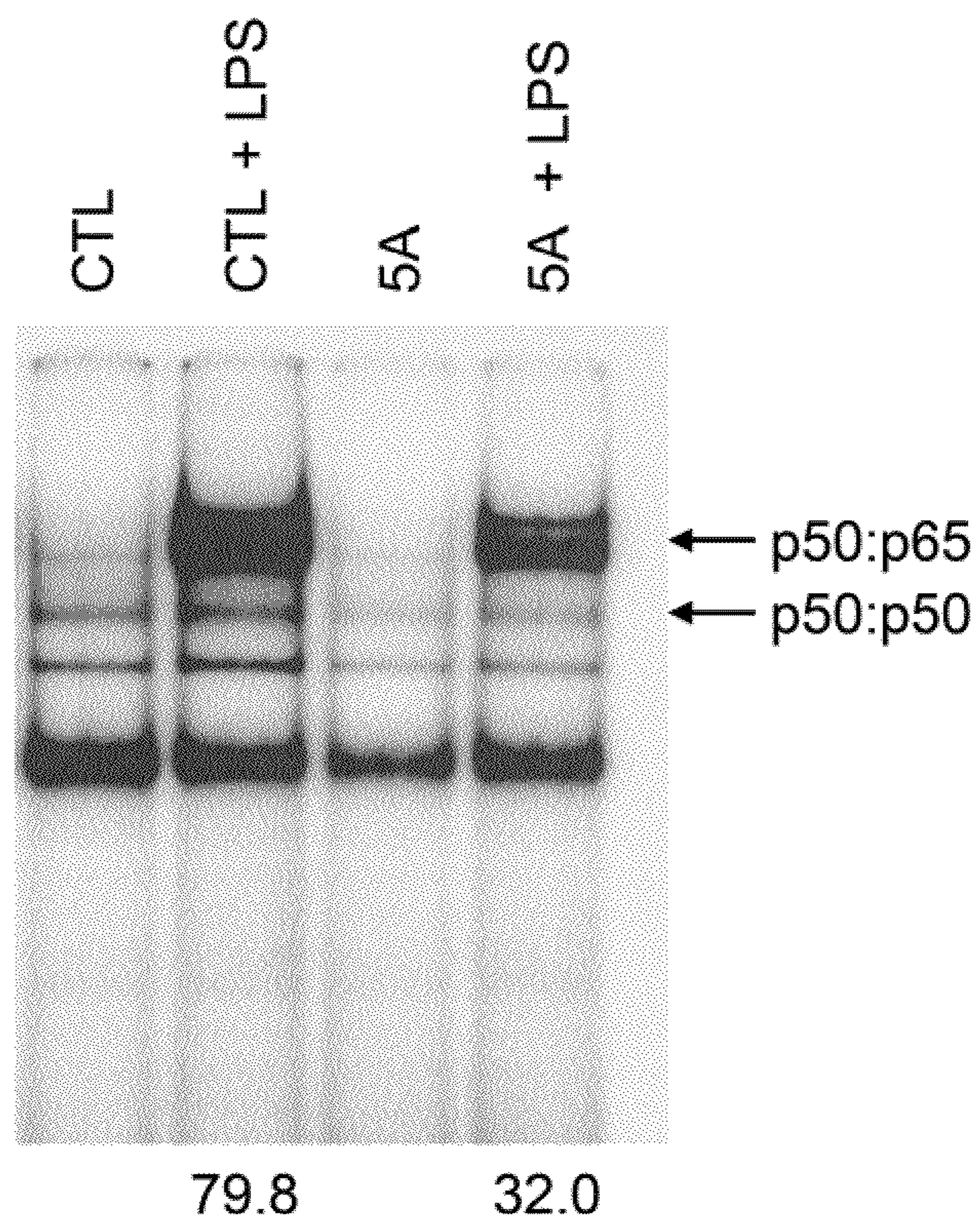
FIG.7C



Pre-treatment with eIF5A1 siRNA #1 prior to Adenovirus infection reduces expression of phosphorylated NF- $\kappa$ B and ICAM-1 in human multiple myeloma cells

FIG.7D

NF-kB DENSITOMETRY SCAN



siRNA-mediated suppression of eIF5A in human multiple myeloma Cells inhibits LPS-mediated induction of NFkB DNA-Binding Activity

FIG. 7E

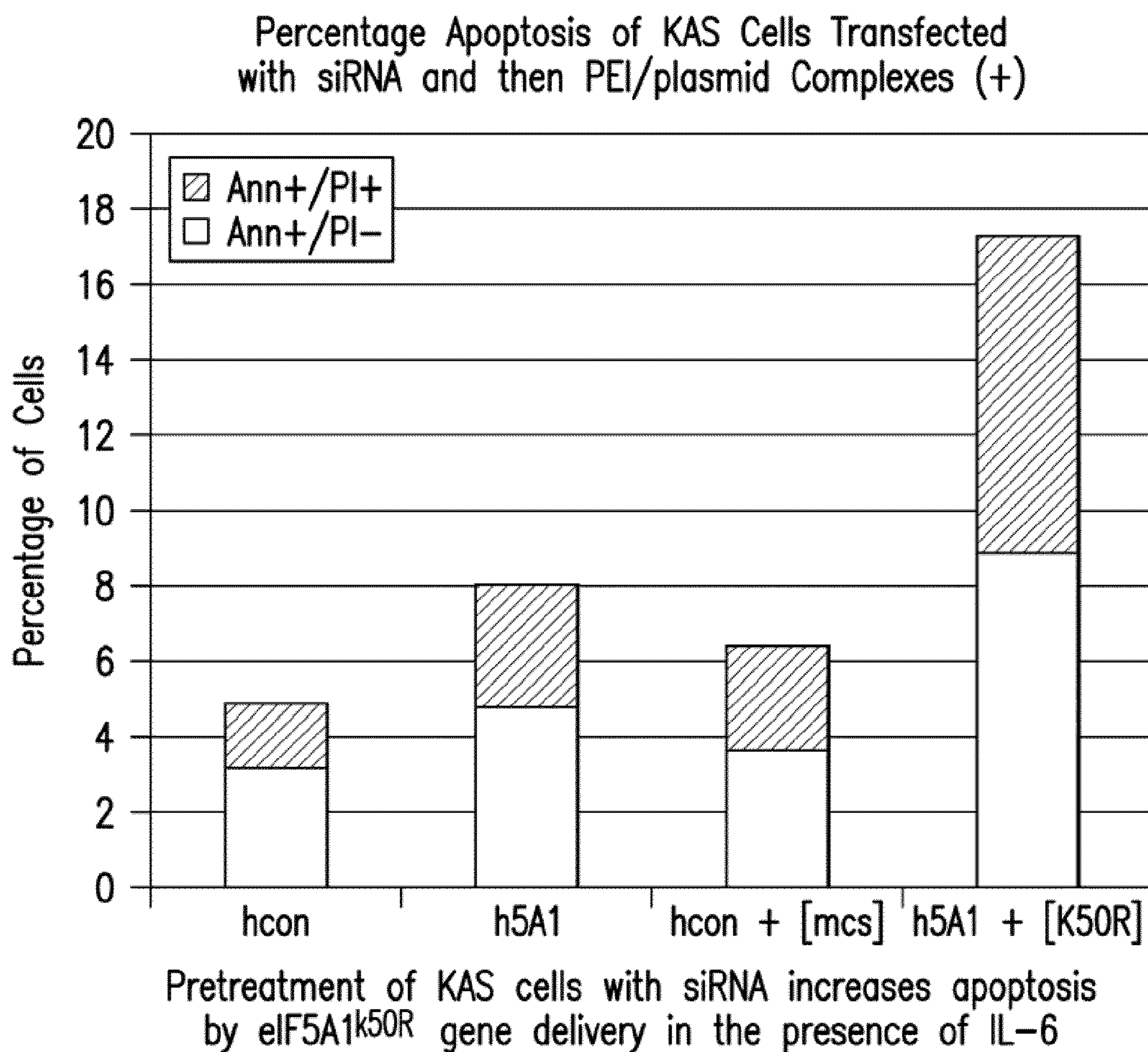


FIG. 7F

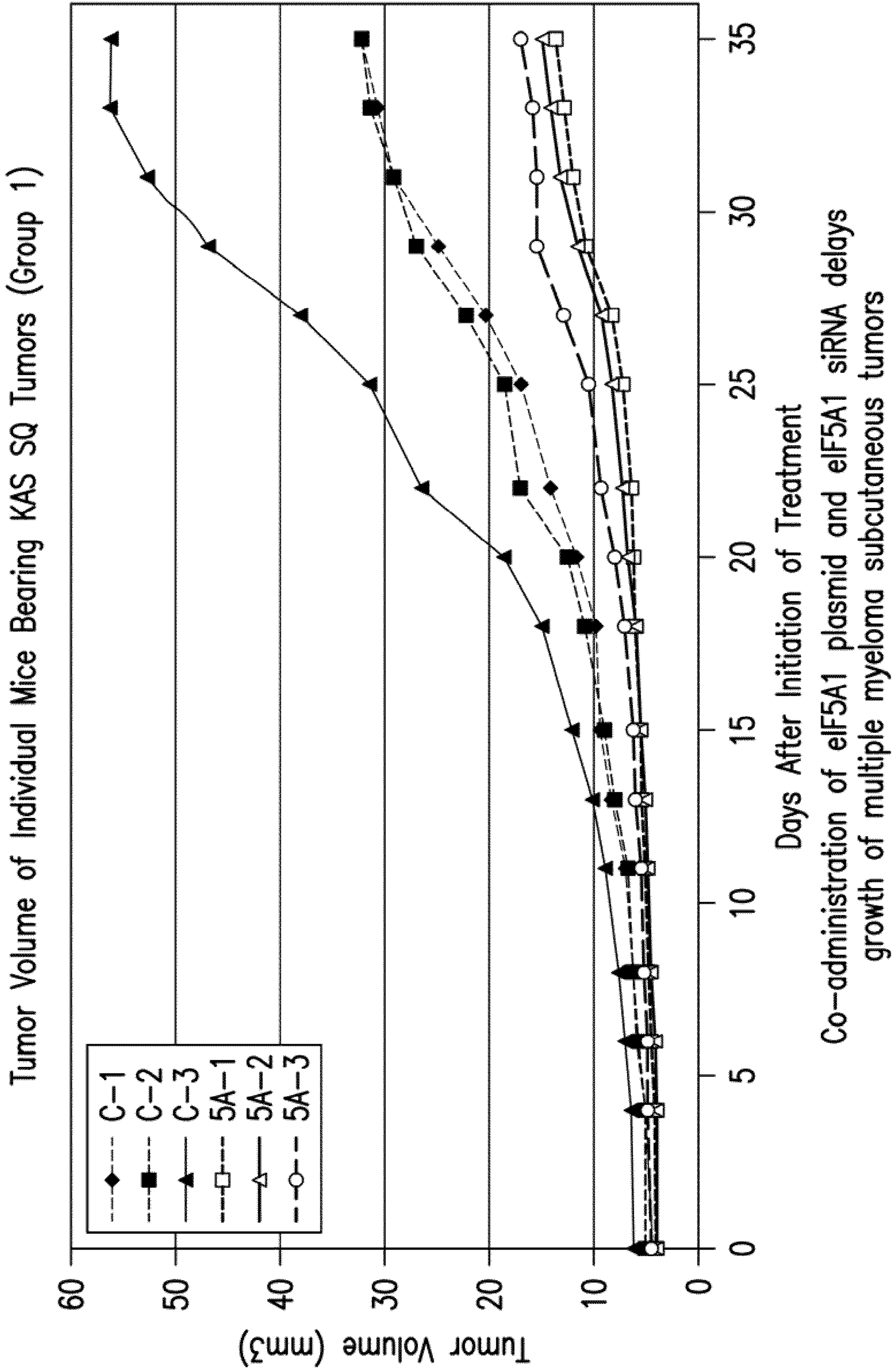


FIG.8



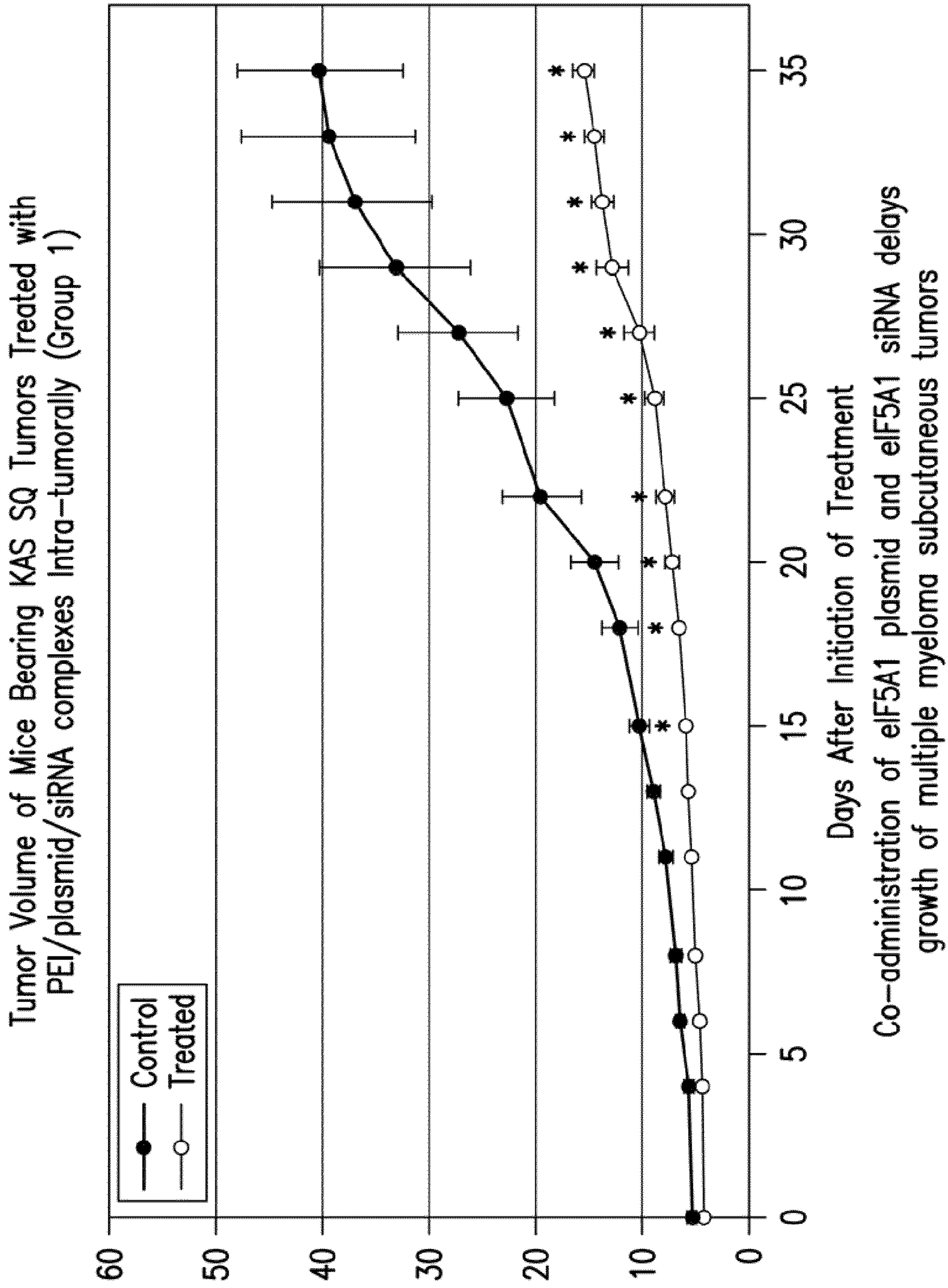


FIG.9

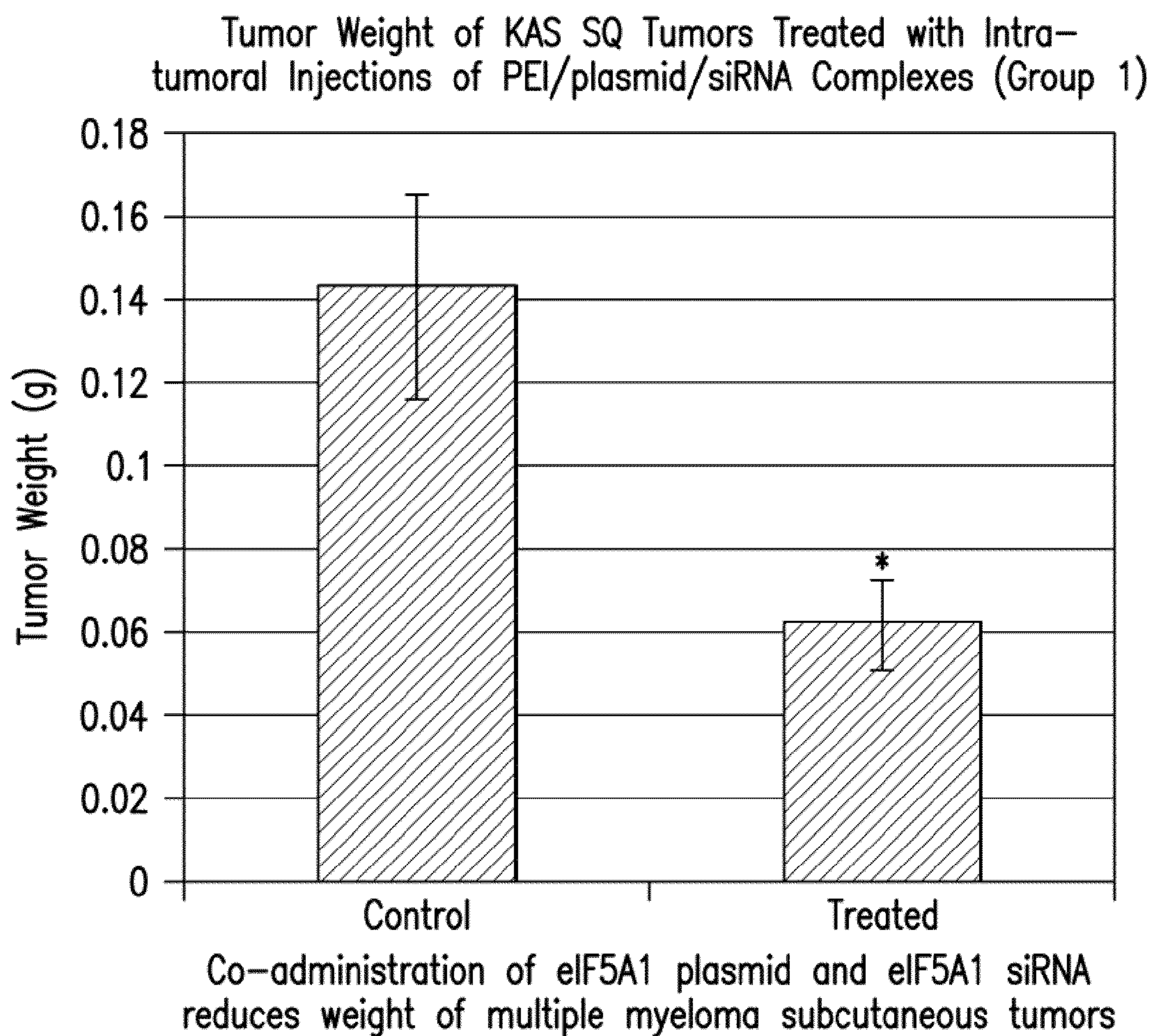
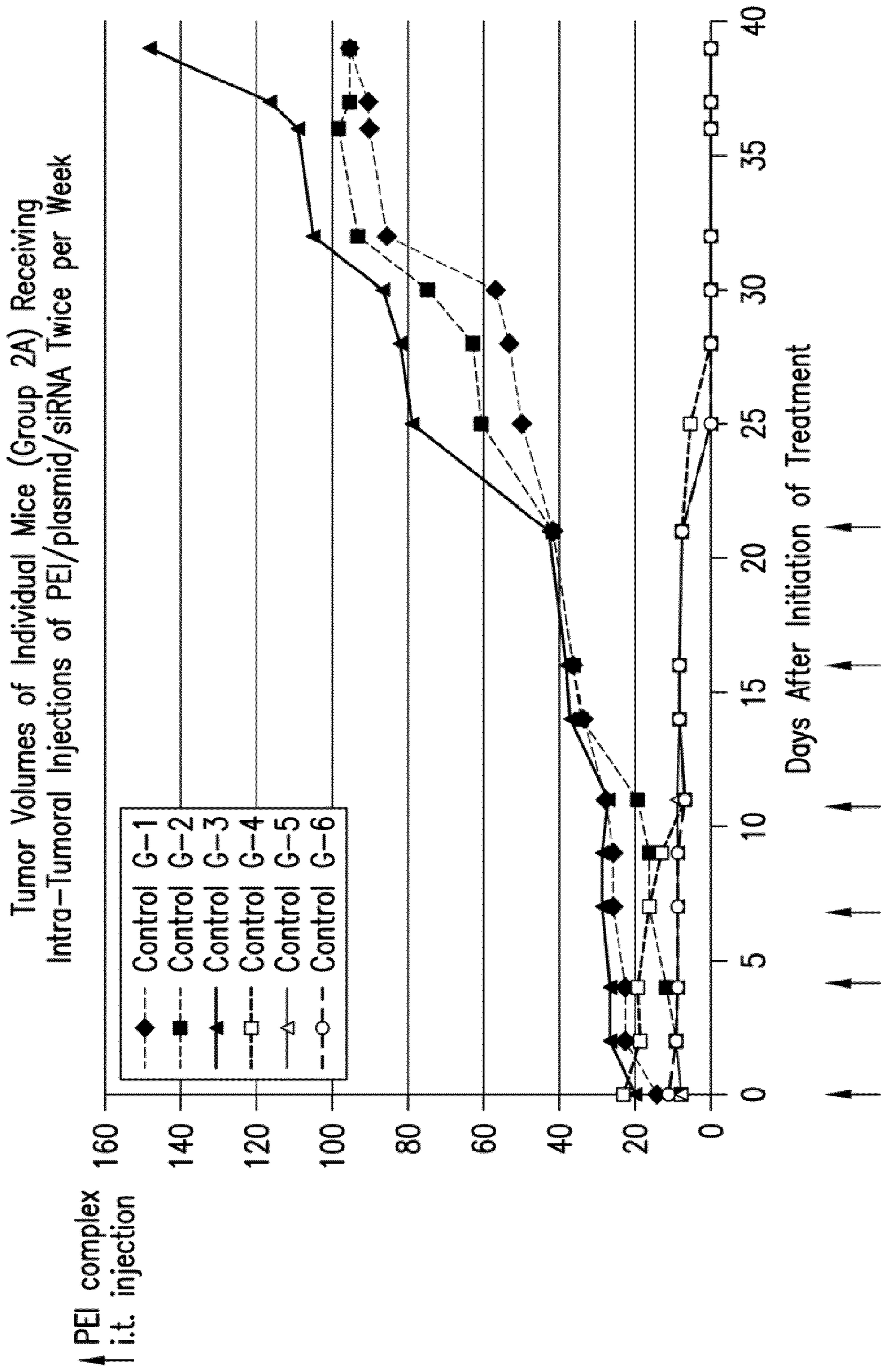
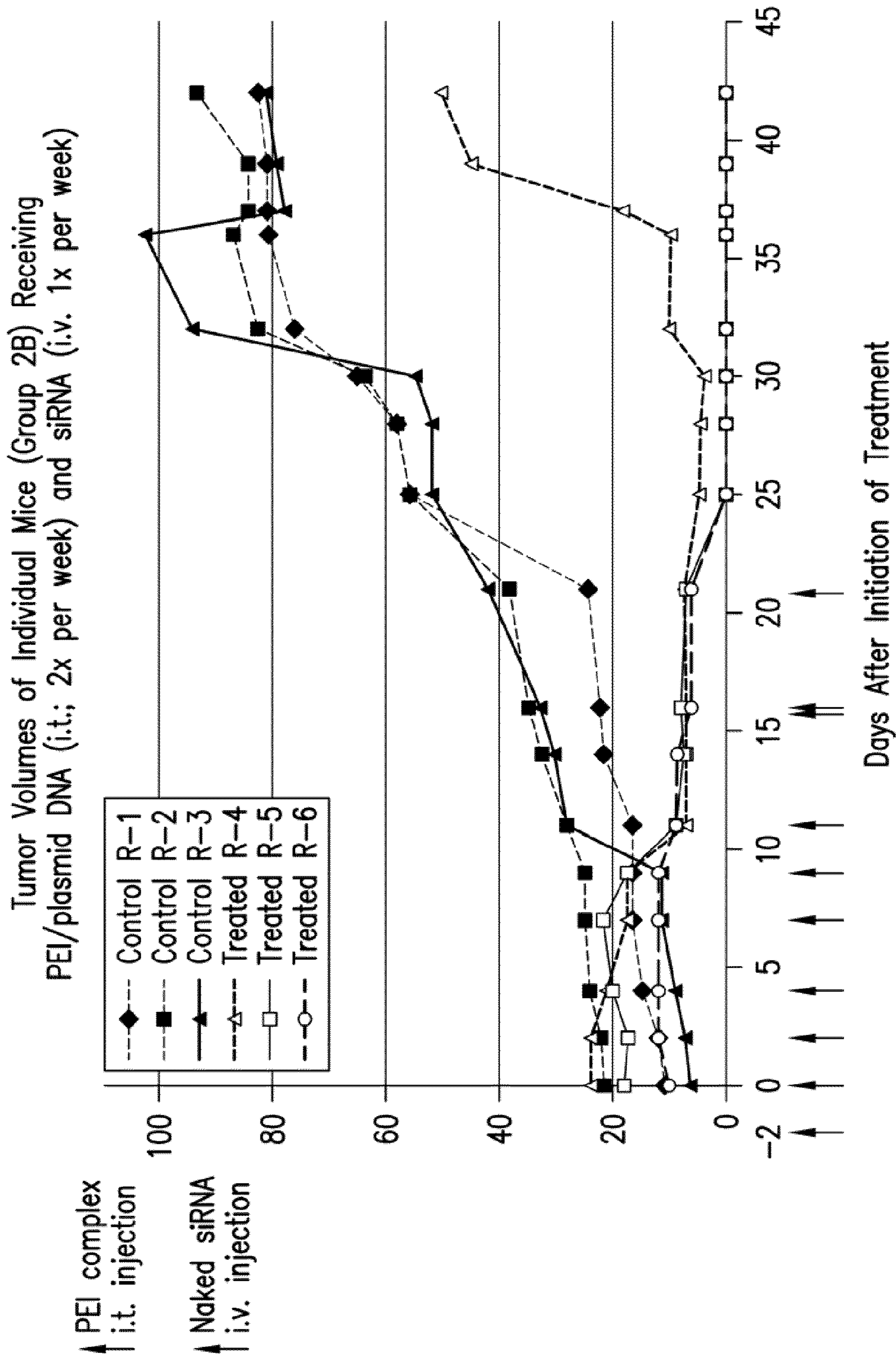


FIG.10



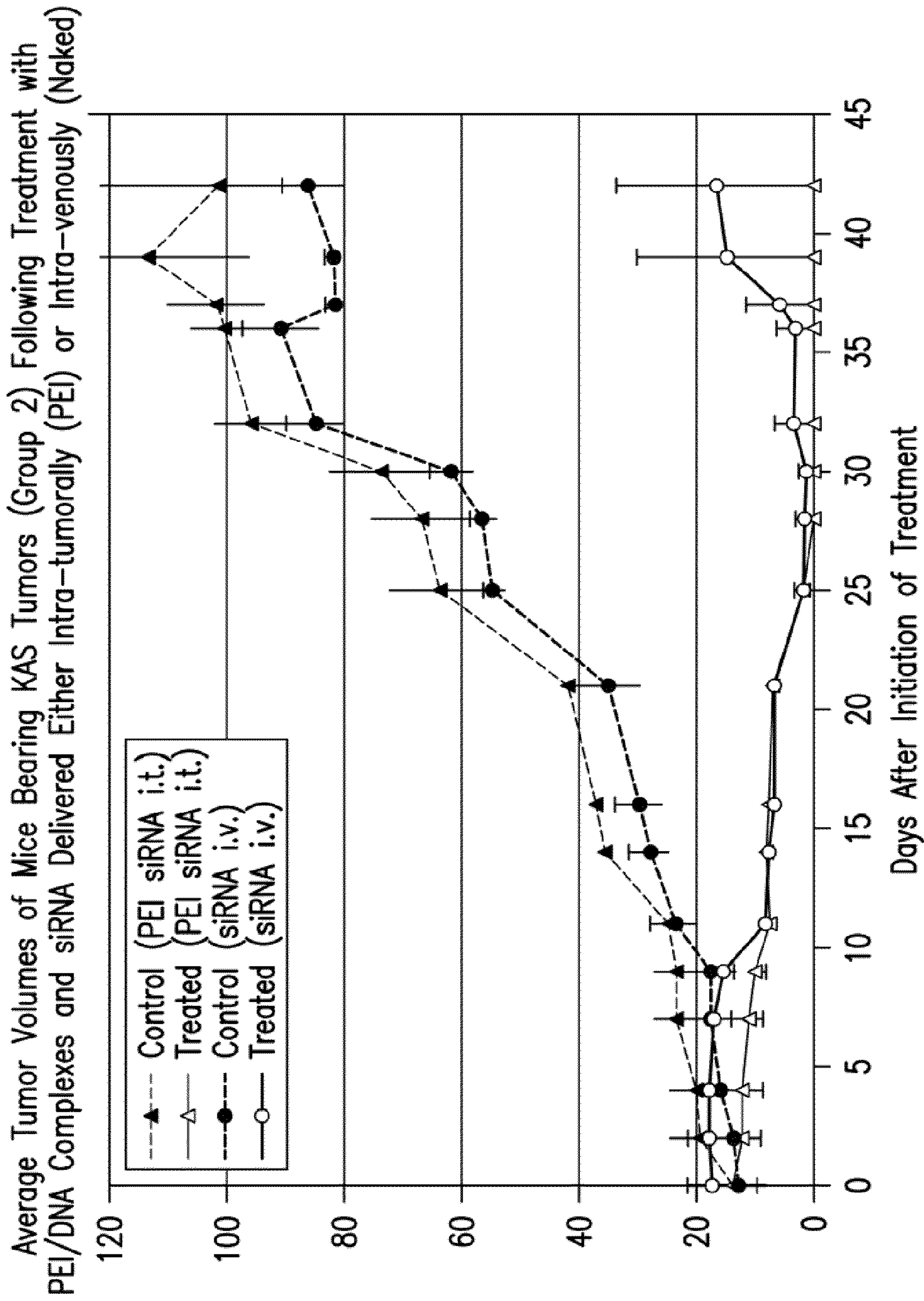
Co-administration of eIF5A1 plasmid and eIF5A1 siRNA inhibits growth of multiple myeloma subcutaneous tumors and results in tumor shrinkage (Group 2A).

FIG.11



Administration of eIF5A1 siRNA intra-venously (i.v.) and PEI/eIF5A1K50R plasmid complexes intra-tumorally (i.t.) results in delay of growth of multiple myeloma subcutaneous tumors and tumor shrinkage (Group 2B).

FIG.12



Treatment with eIF5A1 plasmid and eIF5A1 siRNA delays growth of multiple myeloma subcutaneous tumors and results in tumor shrinkage (Group 2A and 2B).

FIG.13A



Group 2A - mouse G-6



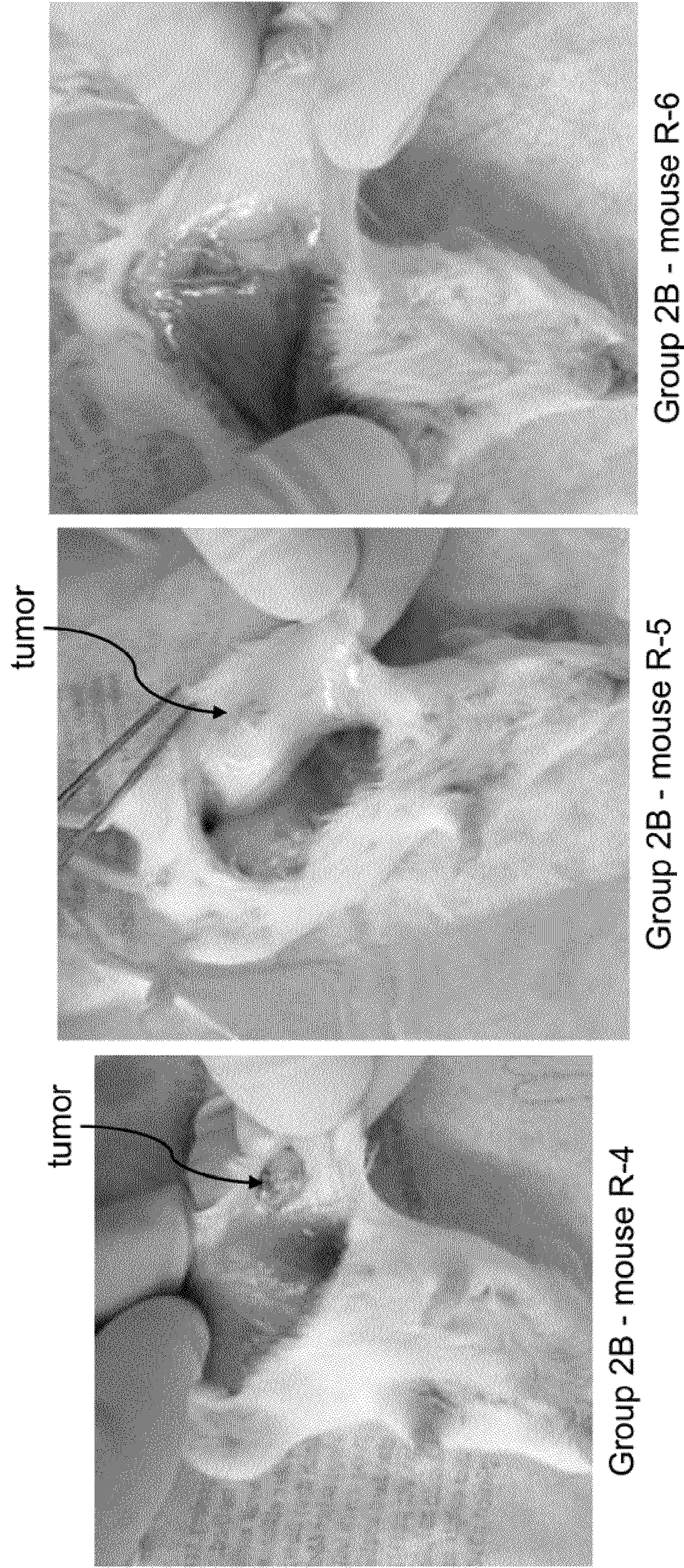
Group 2A - mouse G-5



Group 2A - mouse G-4

Co-administration of eIF5A1 plasmid and eIF5A1 siRNA results in tumor shrinkage (Group 2A).  
No tumor growth was observed in any of the group 2A treated mice.

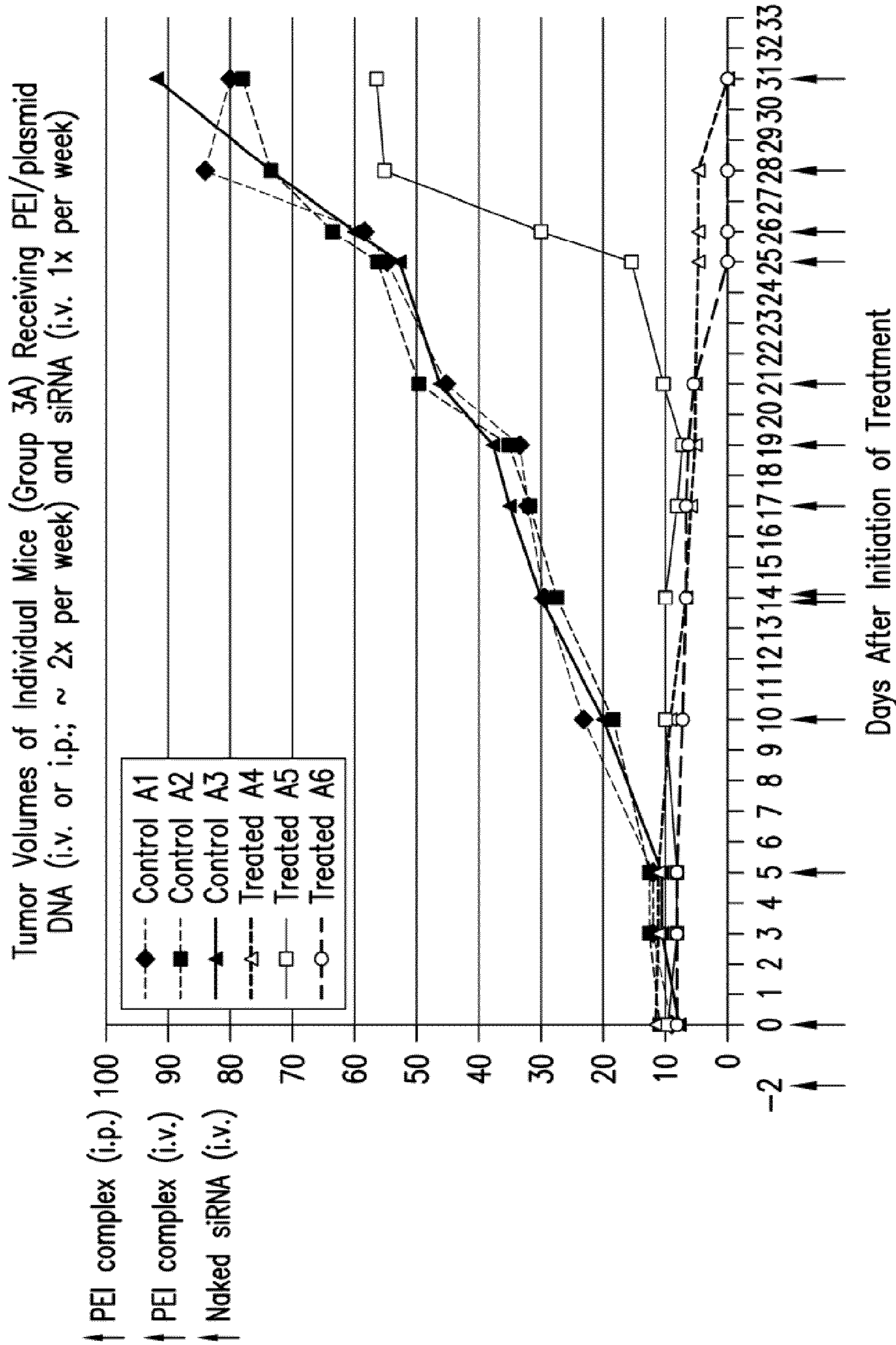
**FIG. 13B**



Group 2B - mouse R-4  
 Group 2B - mouse R-5  
 Group 2B - mouse R-6

Administration of eIF5A1 siRNA intra-venously (i.v.) and PEI/eIF5A1<sup>K50R</sup> plasmid complexes intra-tumorally (i.t.) results in tumor shrinkage of multiple myeloma subcutaneous tumors (Group 2B). There was no evidence of tumor growth in one mouse of the treatment group (group 2B).

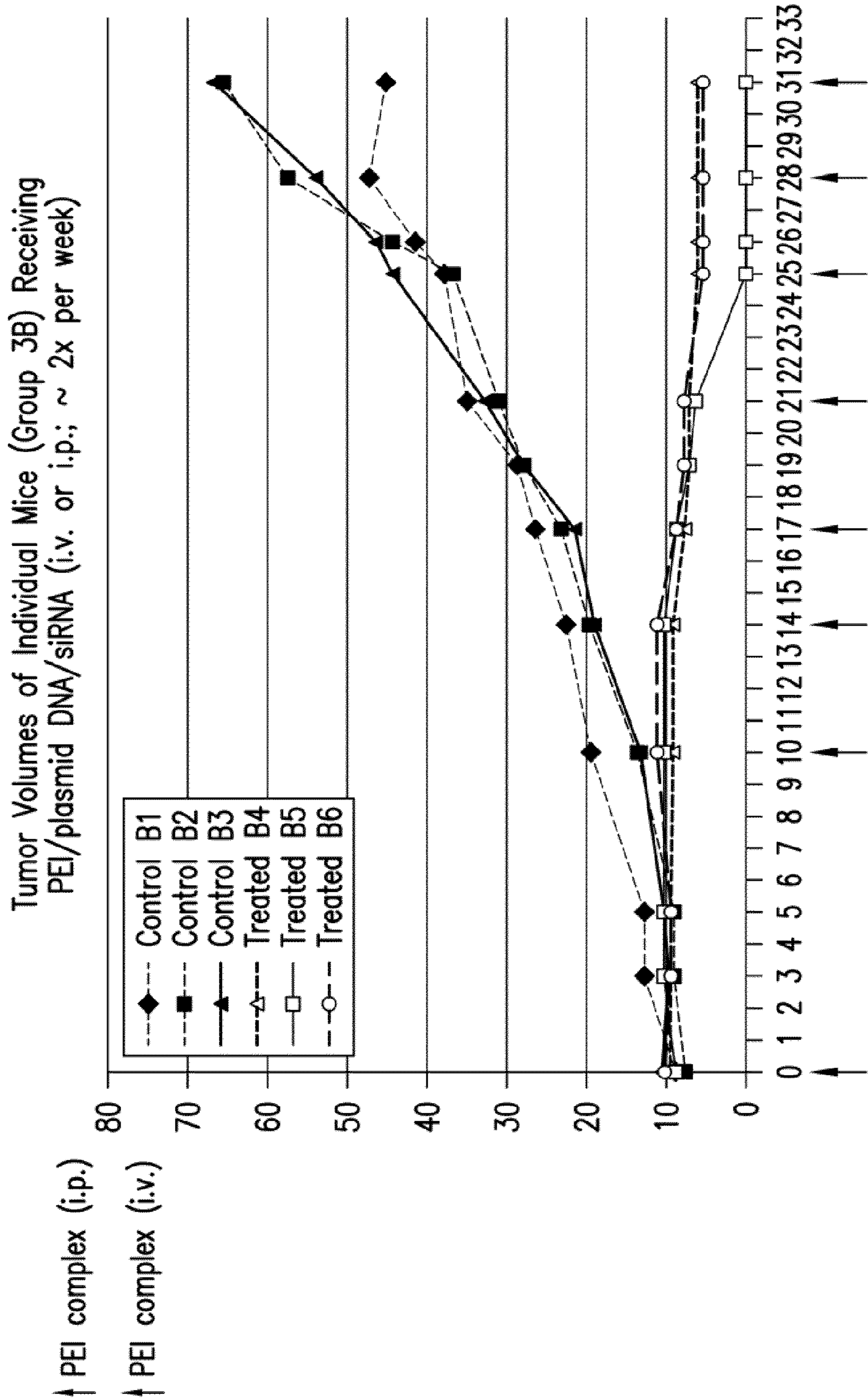
FIG.13C



Administration of eIF5A1 siRNA intra-venously (i.v.) and PEI/eIF5A1K50R plasmid complexes intra-venously (i.v.) or intra-peritoneal (i.p.) delays growth of multiple myeloma subcutaneous tumors (Group 3A)

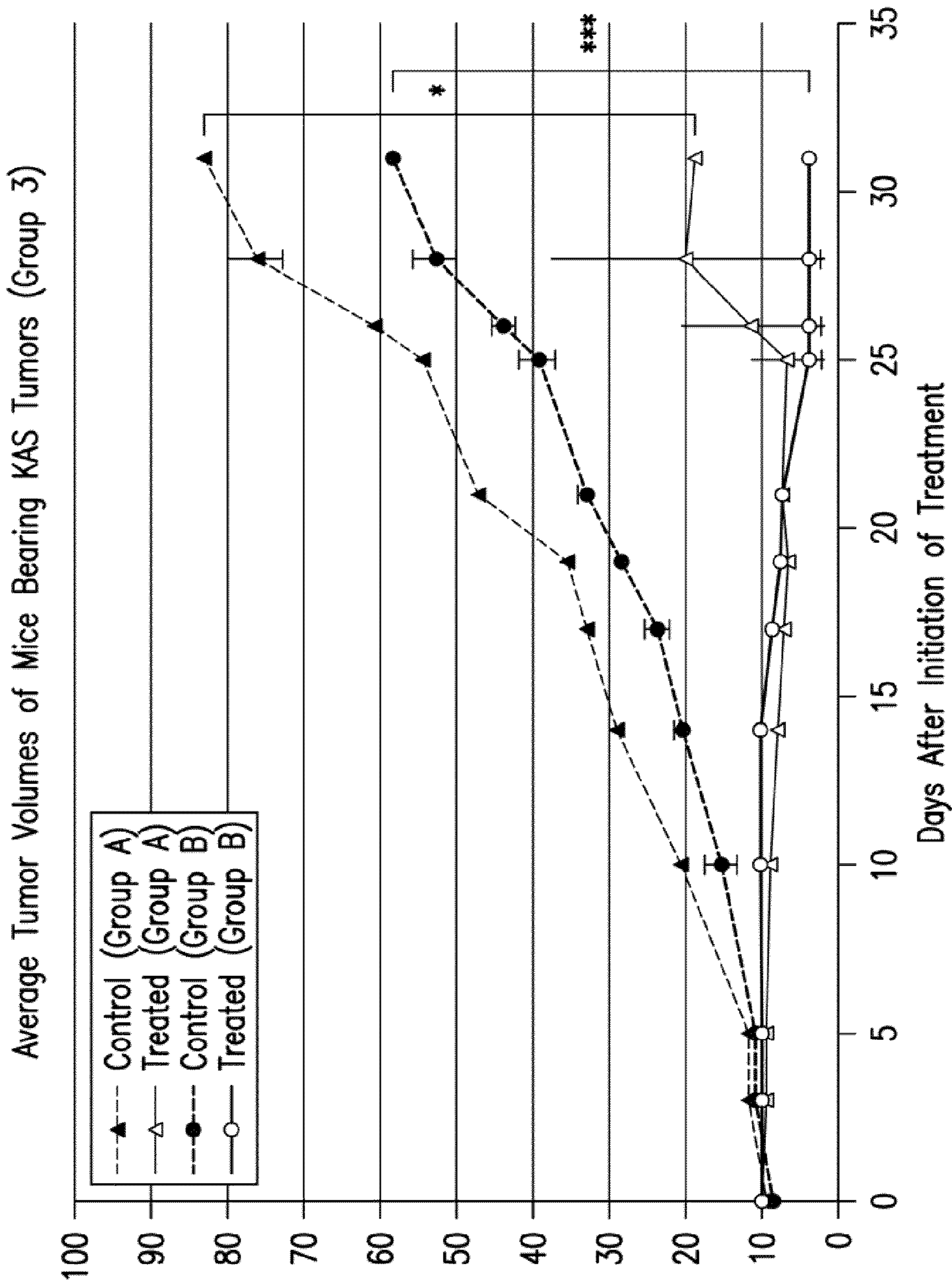
FIG. 14





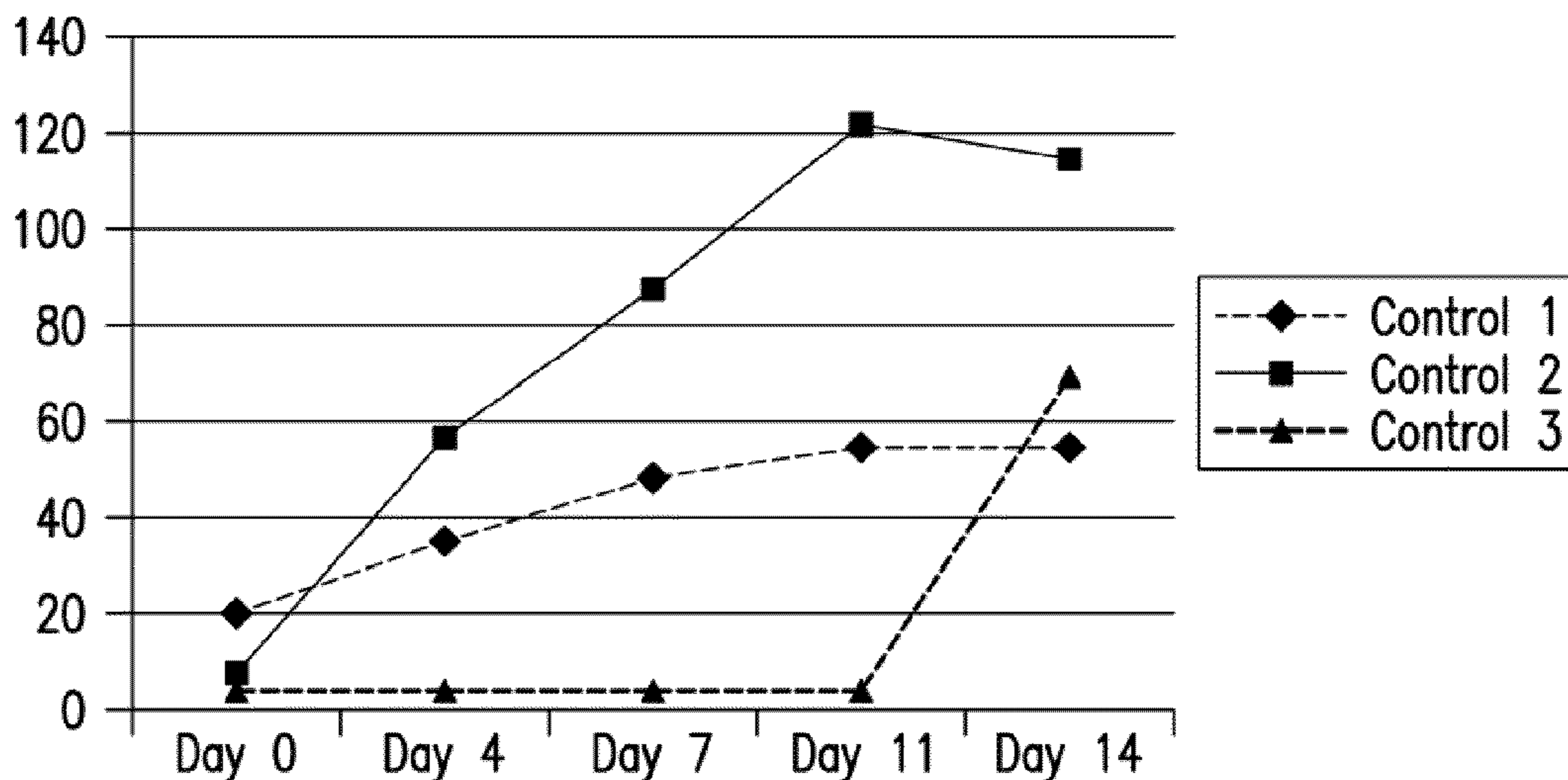
Intra-venous co-administration of eIF5A1 plasmid and eIF5A1 siRNA delays growth of multiple myeloma subcutaneous tumors (Group 3B).

FIG. 15



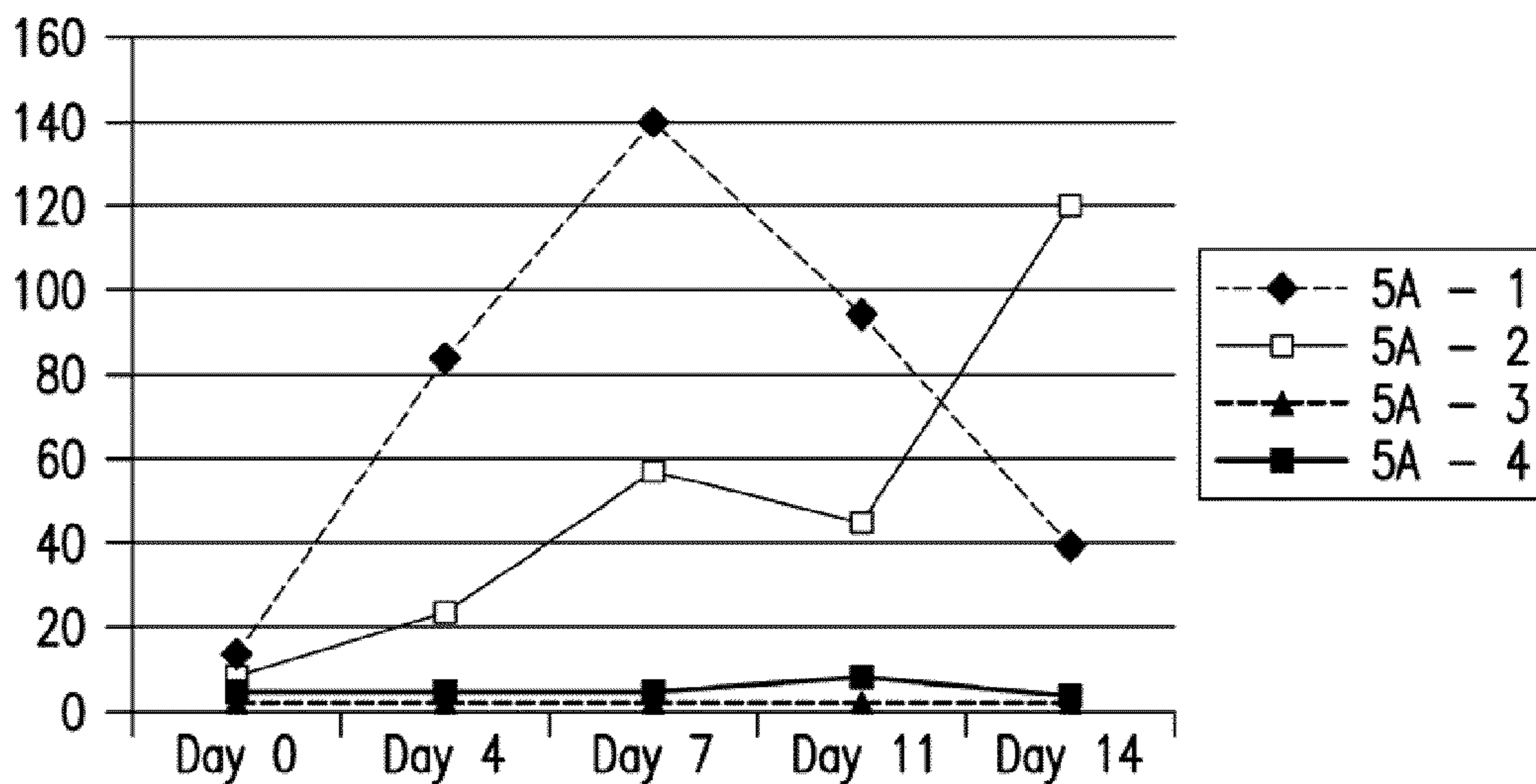
Treatment with eIF5A1 plasmid and eIF5A1 siRNA delays growth of multiple myeloma subcutaneous tumors (Group 3A and 3B).

FIG.16



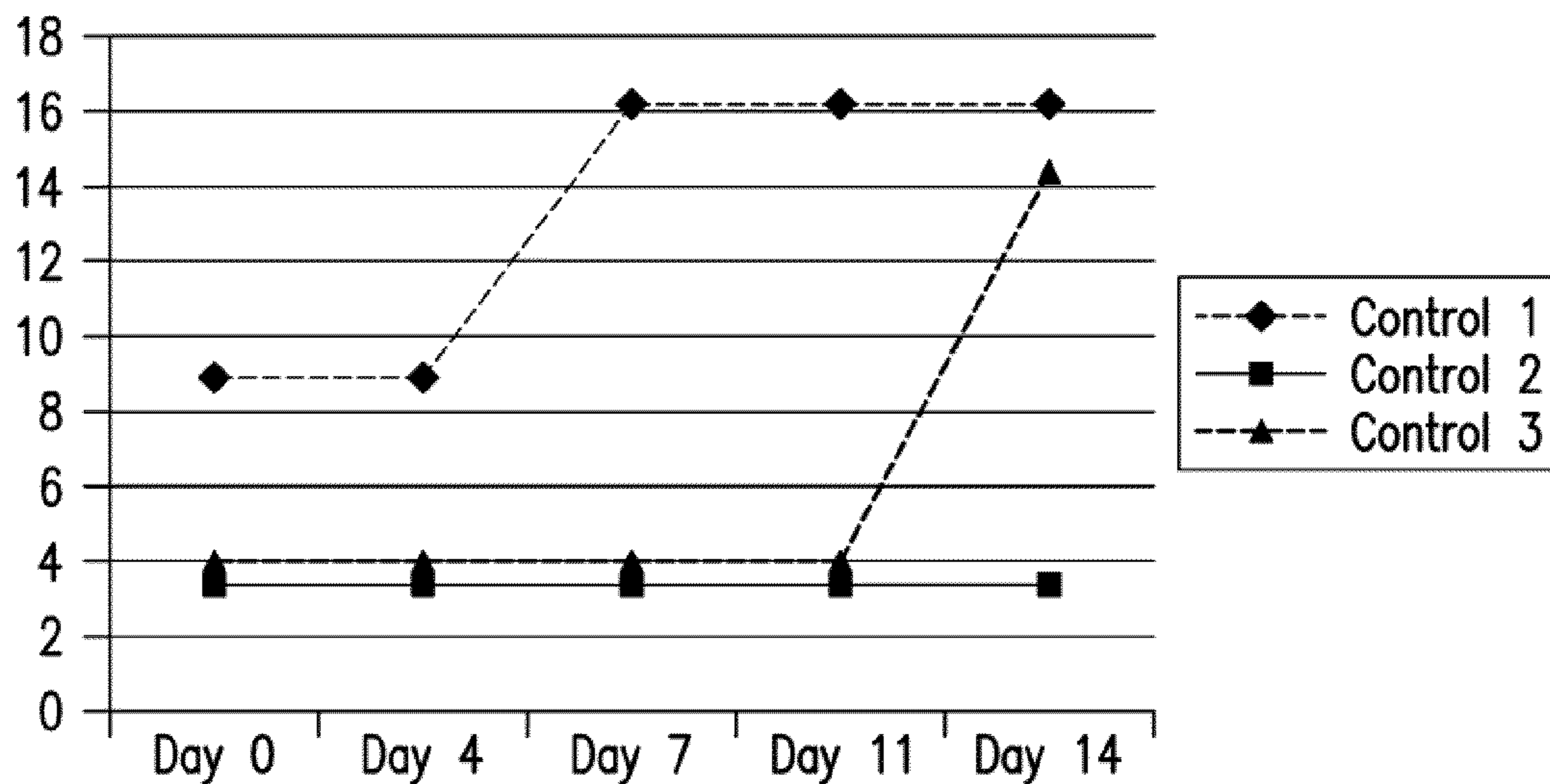
Co-administration of eIF5A1 plasmid and eIF5A1 siRNA delays growth of multiple myeloma subcutaneous tumors and results in tumor shrinkage (Group A).

FIG.17A



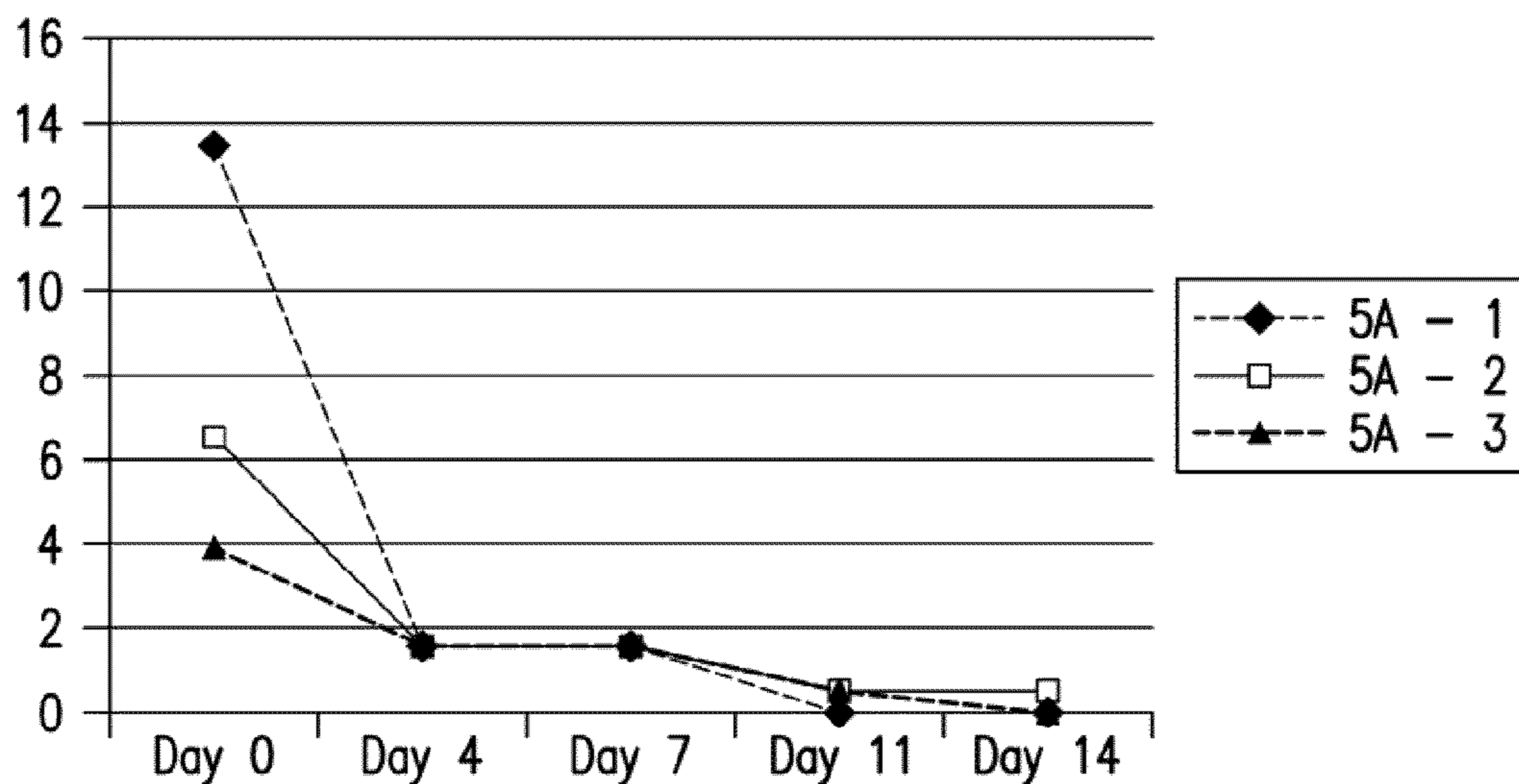
Co-administration of eIF5A1 plasmid and eIF5A1 siRNA delays growth of multiple myeloma subcutaneous tumors and results in tumor shrinkage (Group A).

FIG.17B



Administration of eIF5A1 siRNA intra-venously (i.v.) and PEI/eIF5A1<sup>K50R</sup> plasmid complexes intra-tumourally (i.t.) results in tumor shrinkage of multiple myeloma subcutaneous tumors (Group B).

FIG. 18A



Administration of eIF5A1 siRNA intra-venously (i.v.) and PEI/eIF5A1<sup>K50R</sup> plasmid complexes intra-tumourally (i.t.) results in tumor shrinkage of multiple myeloma subcutaneous tumors (Group B).

FIG. 18B

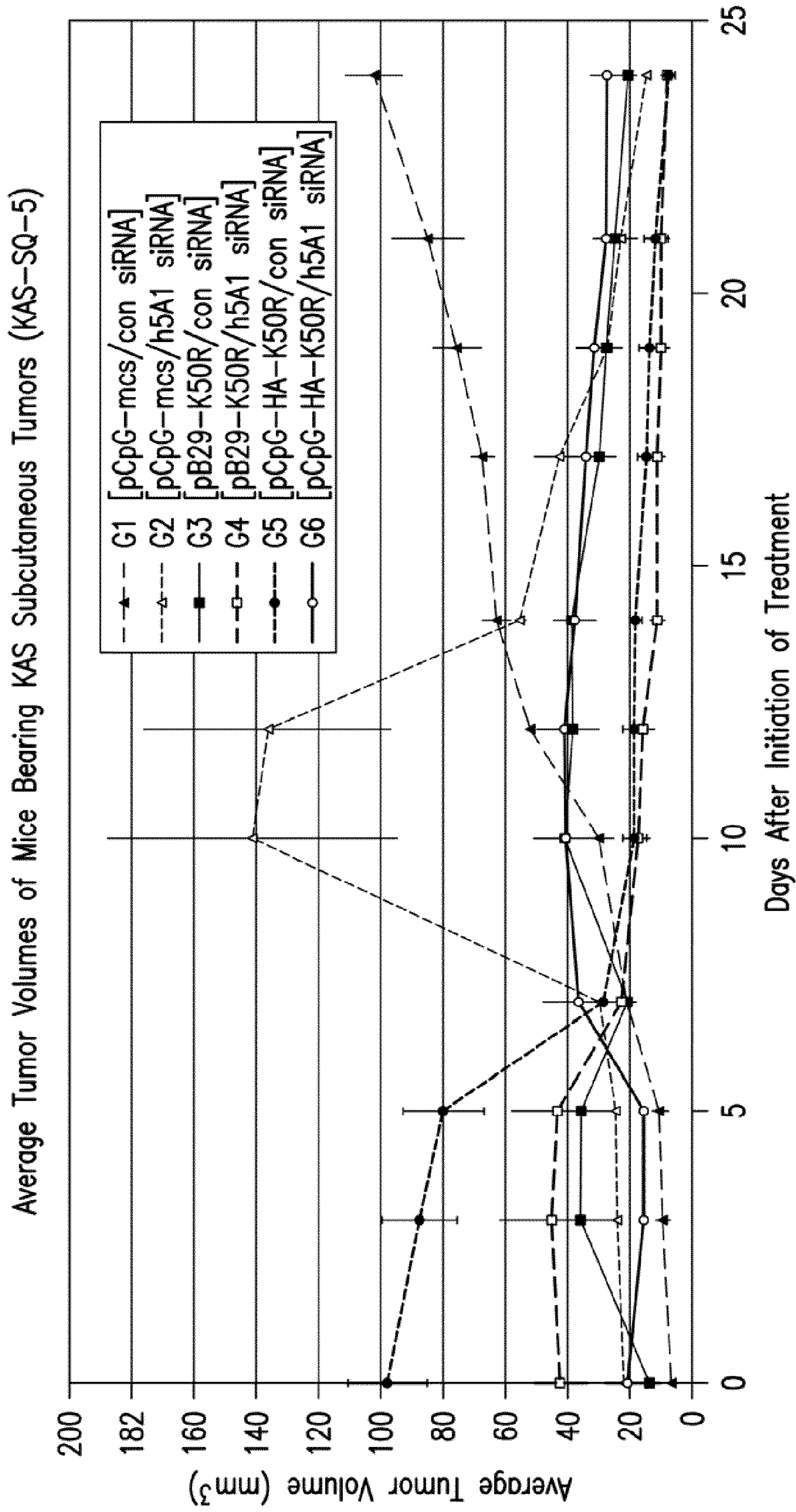
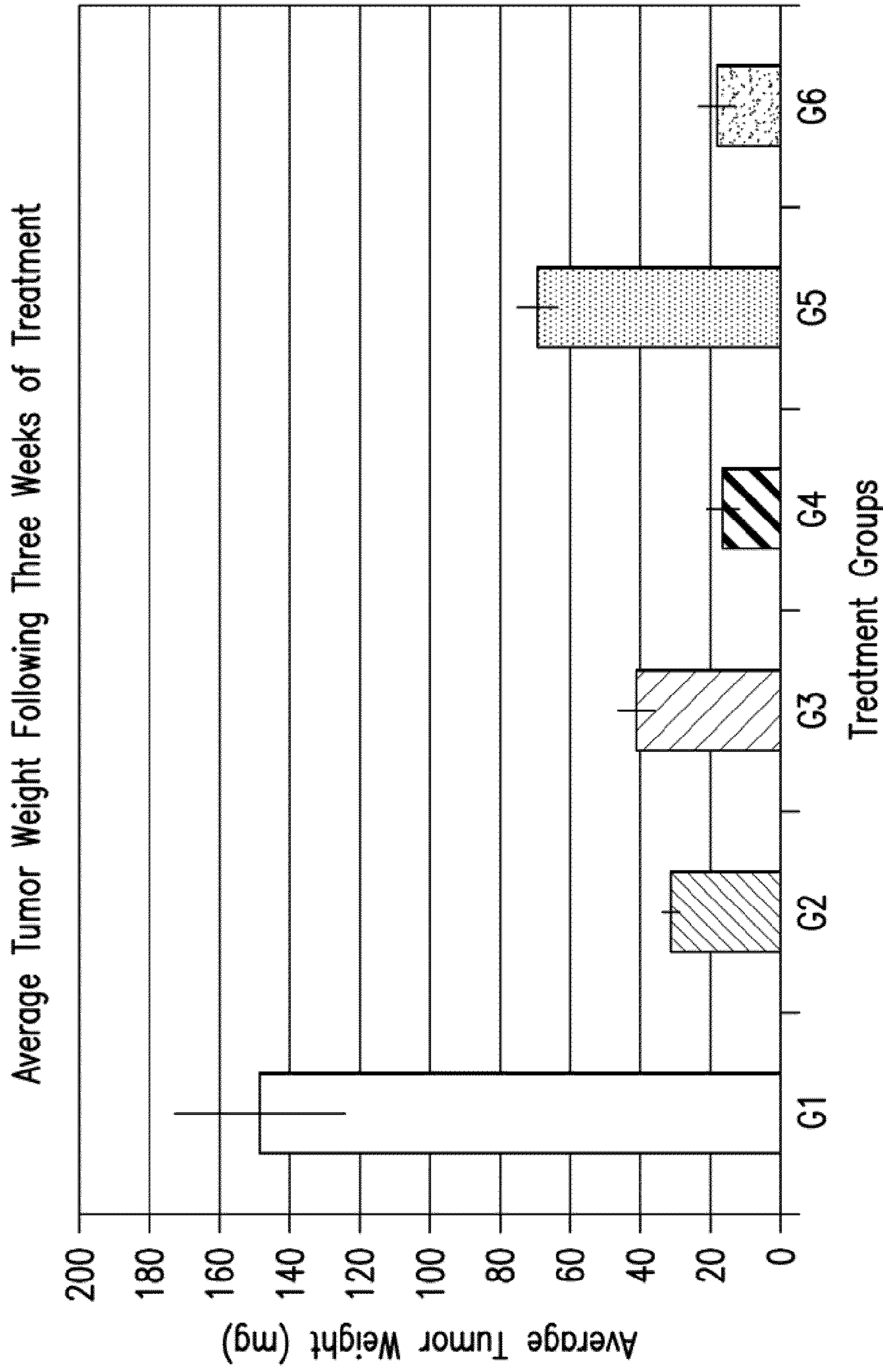
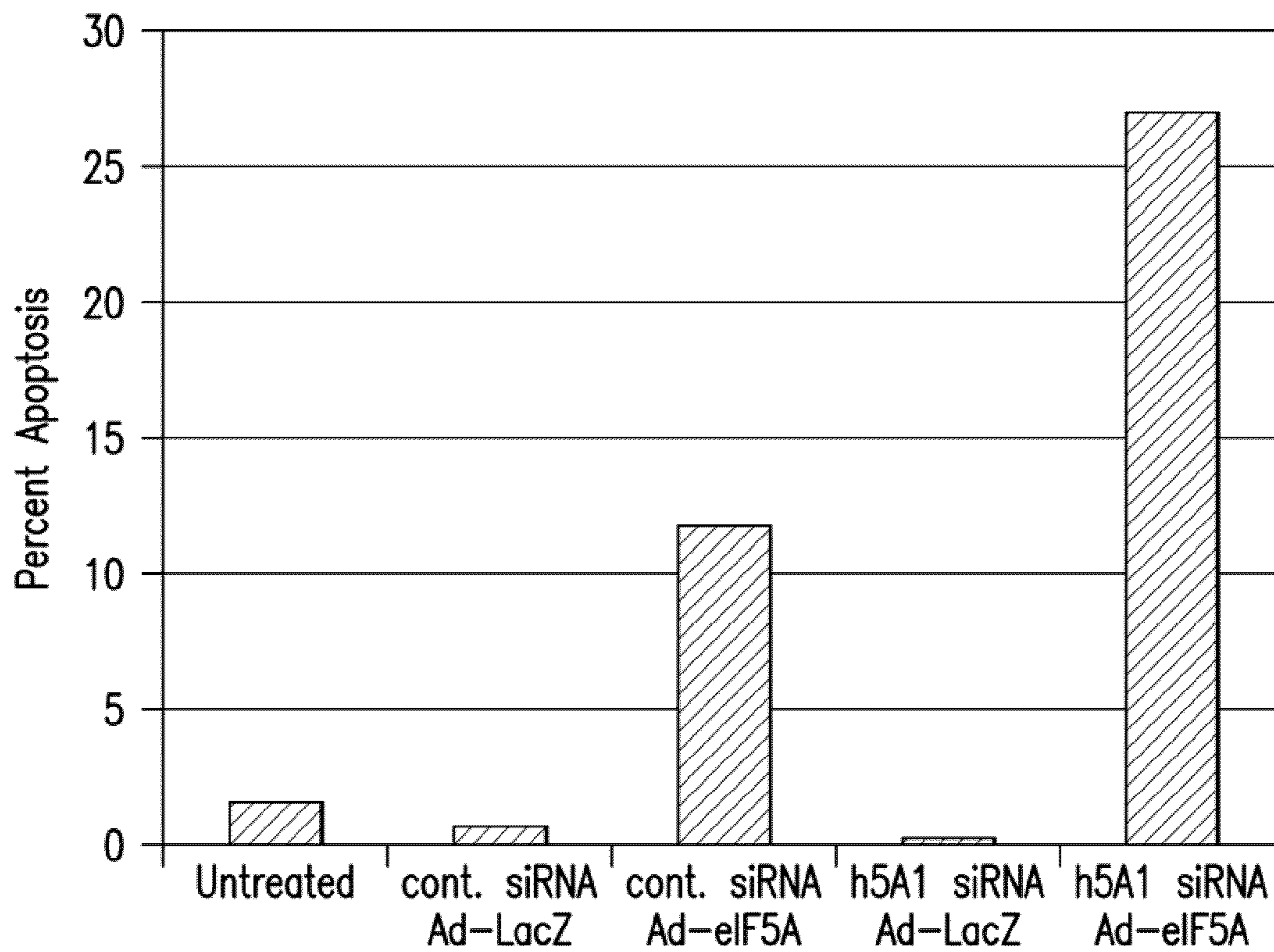


FIG.19



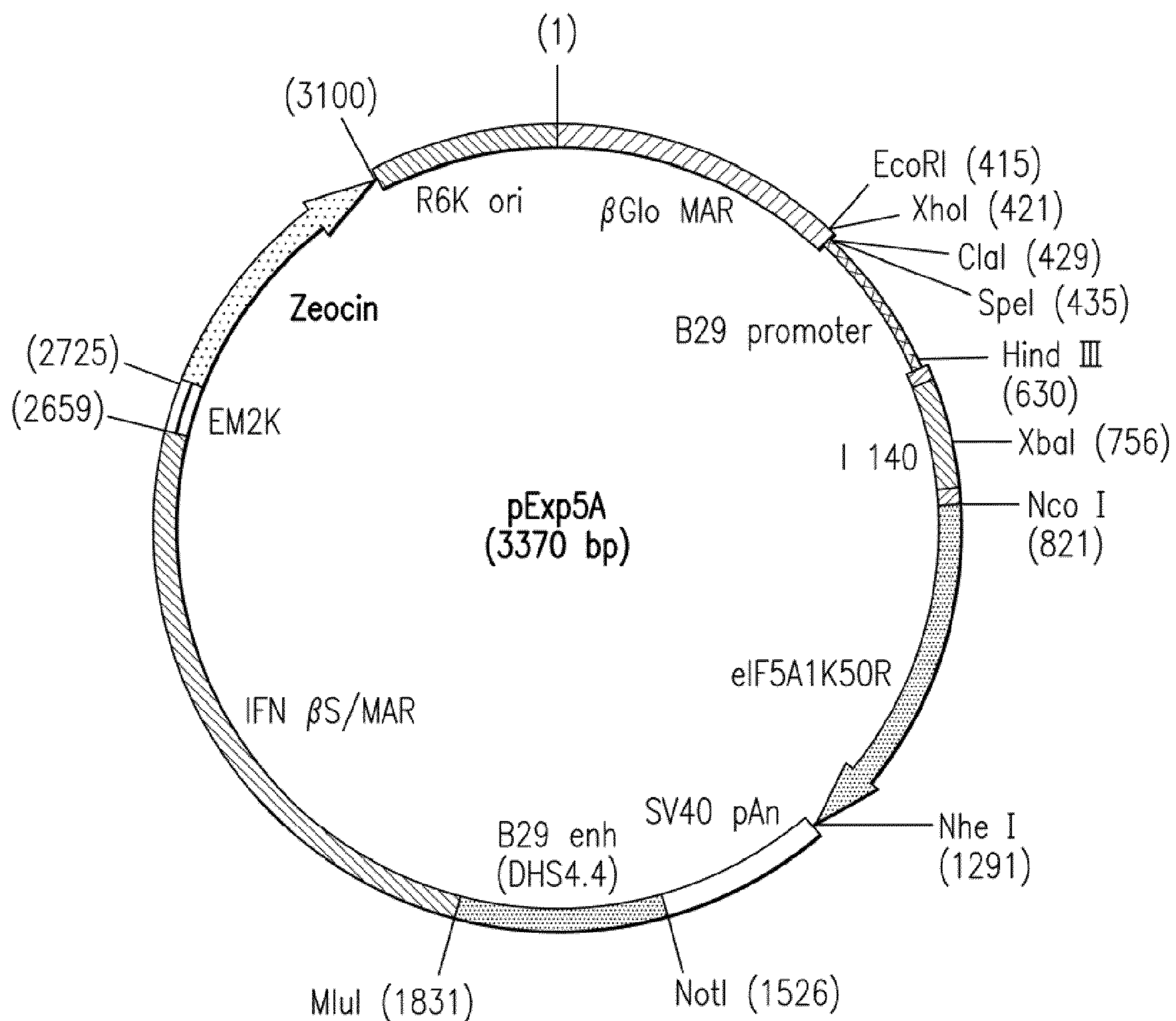
Co-administration of eIF5A1 siRNA increases anti-tumor effect of eIF5A1<sup>K50R</sup> plasmid, driven by either the EF1 or B29 promoter, on multiple myeloma subcutaneous tumors and results in reduced tumor burden (KAS-SQ-5).

FIG. 20



eIF5A1 siRNA Synergistically Increases Apoptosis Induction Resulting from Infection with Ad-eIF5A in Lung Adenocarcinoma Cells

FIG.21



Note: All the labeled restriction sites are unique

FIG.22



Predicted Sequence of pExp5A (3371 bp)

Single-cutting Restriction Enzyme sites are highlighted

EcoRI = GAATTC; XhoI = CTCGAG; ClaI = ATCGAT ; SpeI = ACTAGT;  
HindIII = AAGCTT; NcoI = CCATGG; NheI = GCTAGC; MluI = ACGCGT

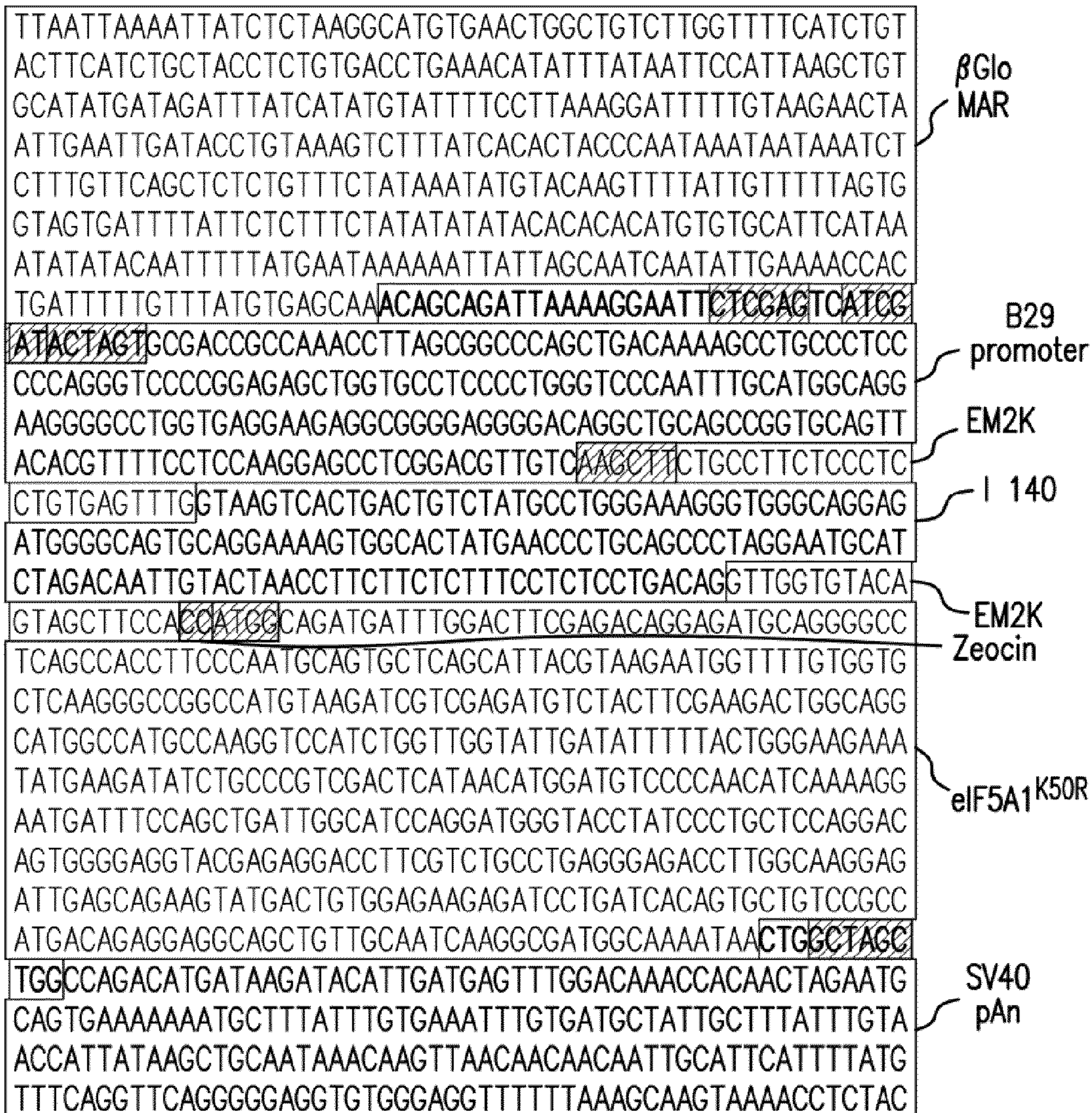


FIG.23A

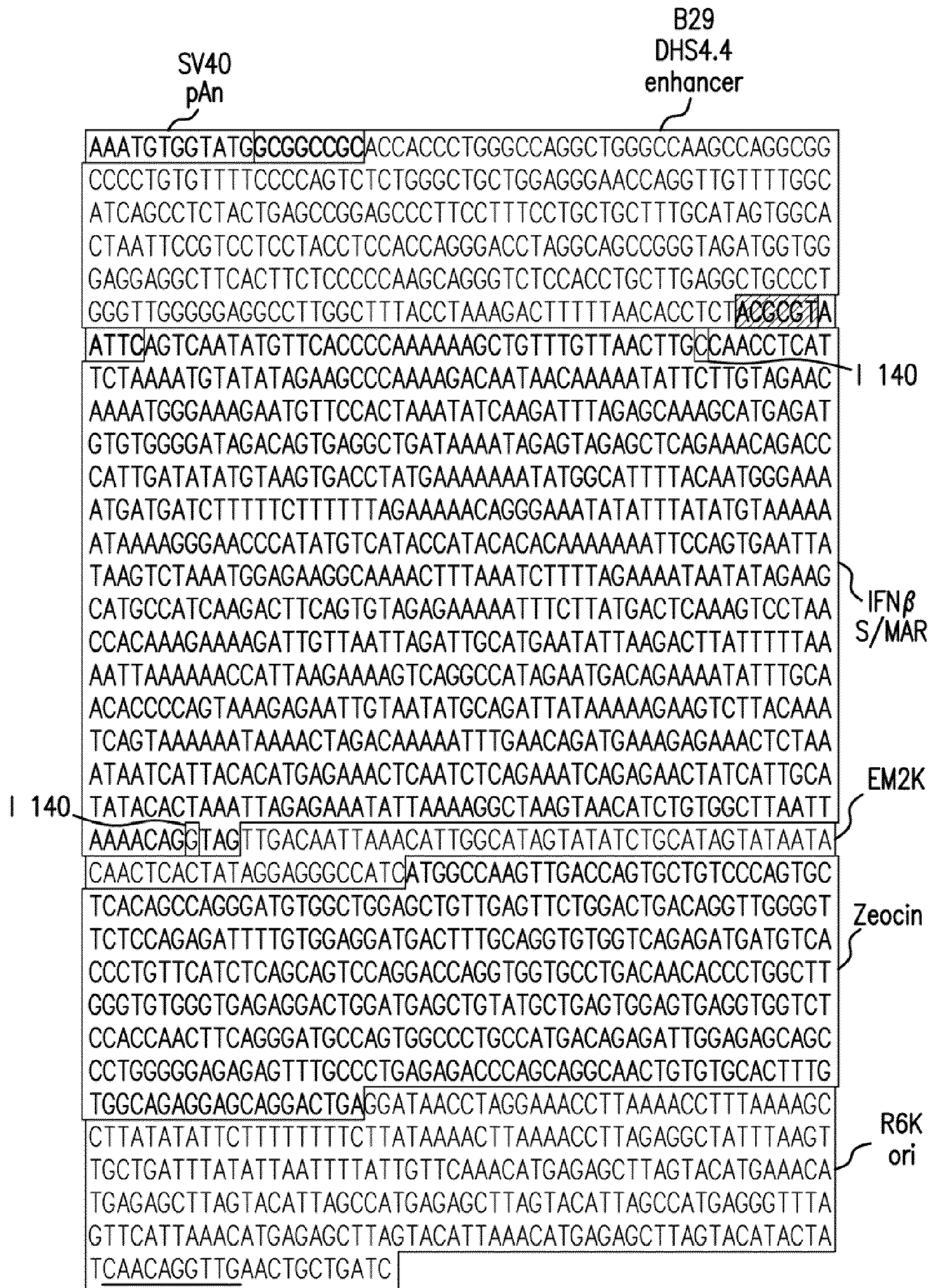


FIG. 23B

plasmid name	5' enhancer	promoter	3' enhancer	gene
pCpG-HA-K50R	CMV	EF1	none	HA-eIF5A1 <sup>K50R</sup>
B29-HA-K50R	CMV	B29-min	none	HA-eIF5A1 <sup>K50R</sup>
pExp-5	none	B29-min	4.4DHS	HA-eIF5A1 <sup>K50R</sup>
pFexp-1	none	B29-full	4.4DHS	HA-eIF5A1 <sup>K50R</sup>

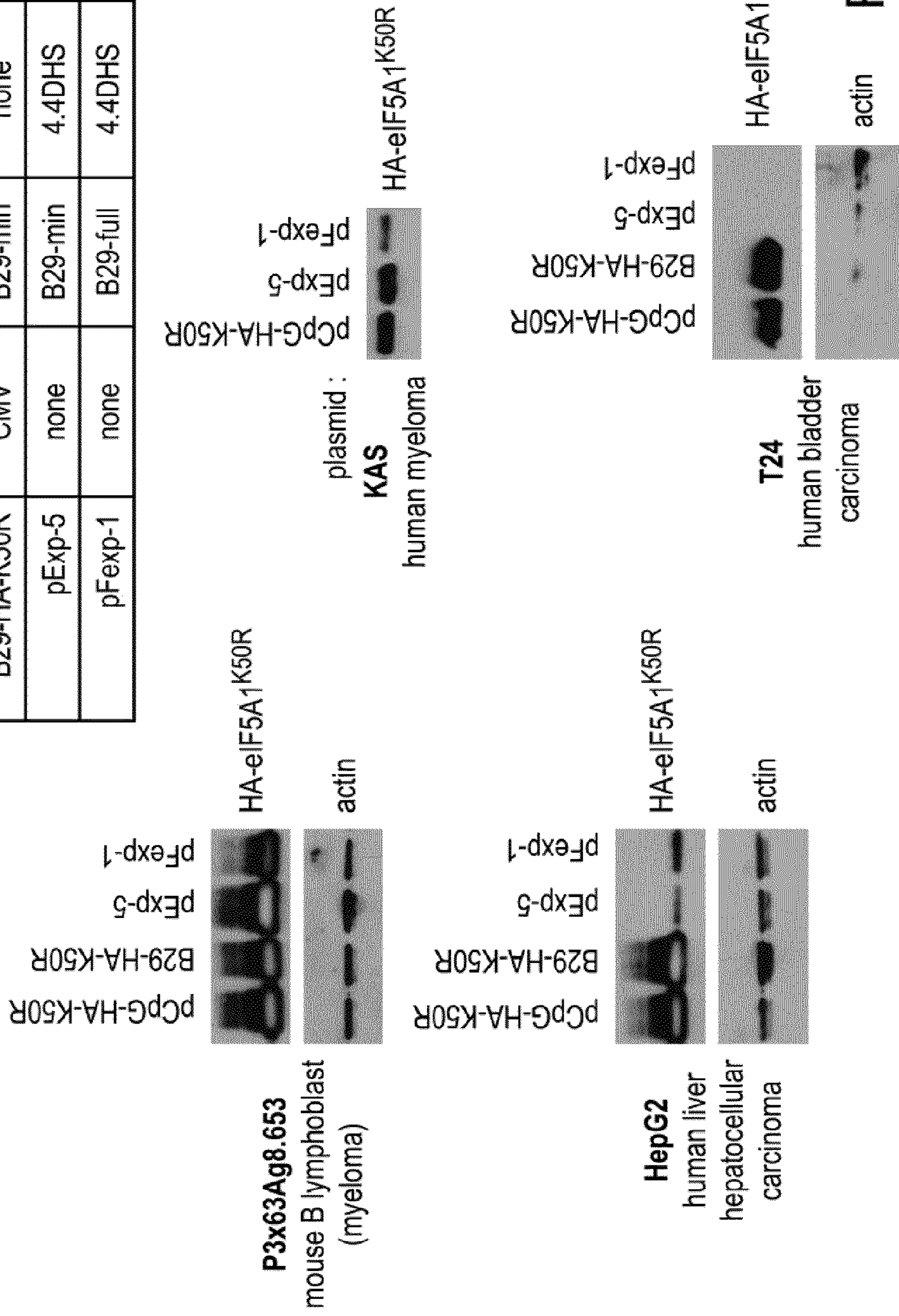
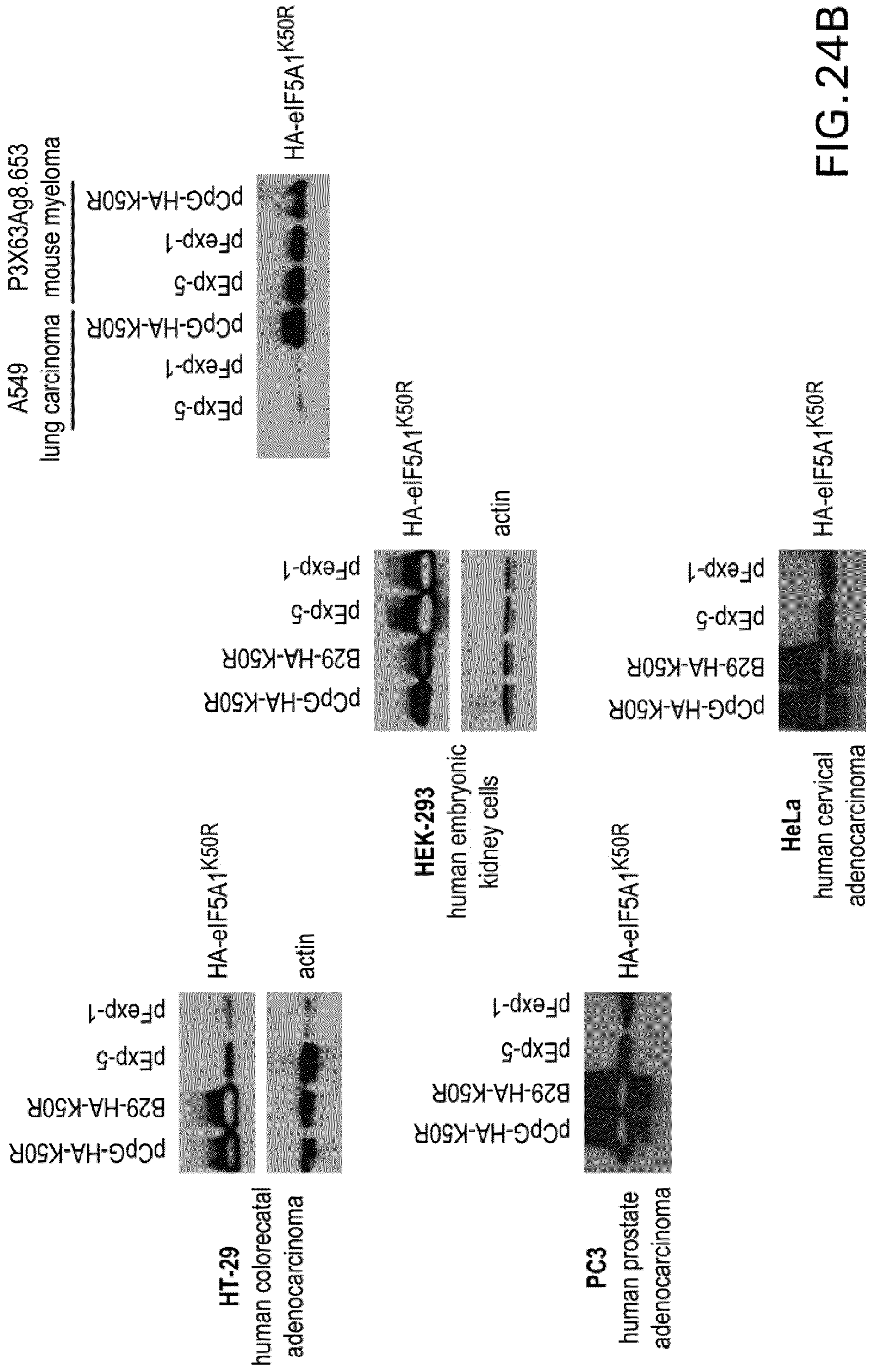


FIG. 24A



h5A1siRNA

Target sequence : 5' GCU GGA CUC CUC CUA CAC A (dTdT) 3'

siRNA

5' GCU GGA CUC CUC CUA CAC A dTdT 3'  
3' dTdT CGA CCU GAG GAG GAU GUGU 5'

**FIG.25**

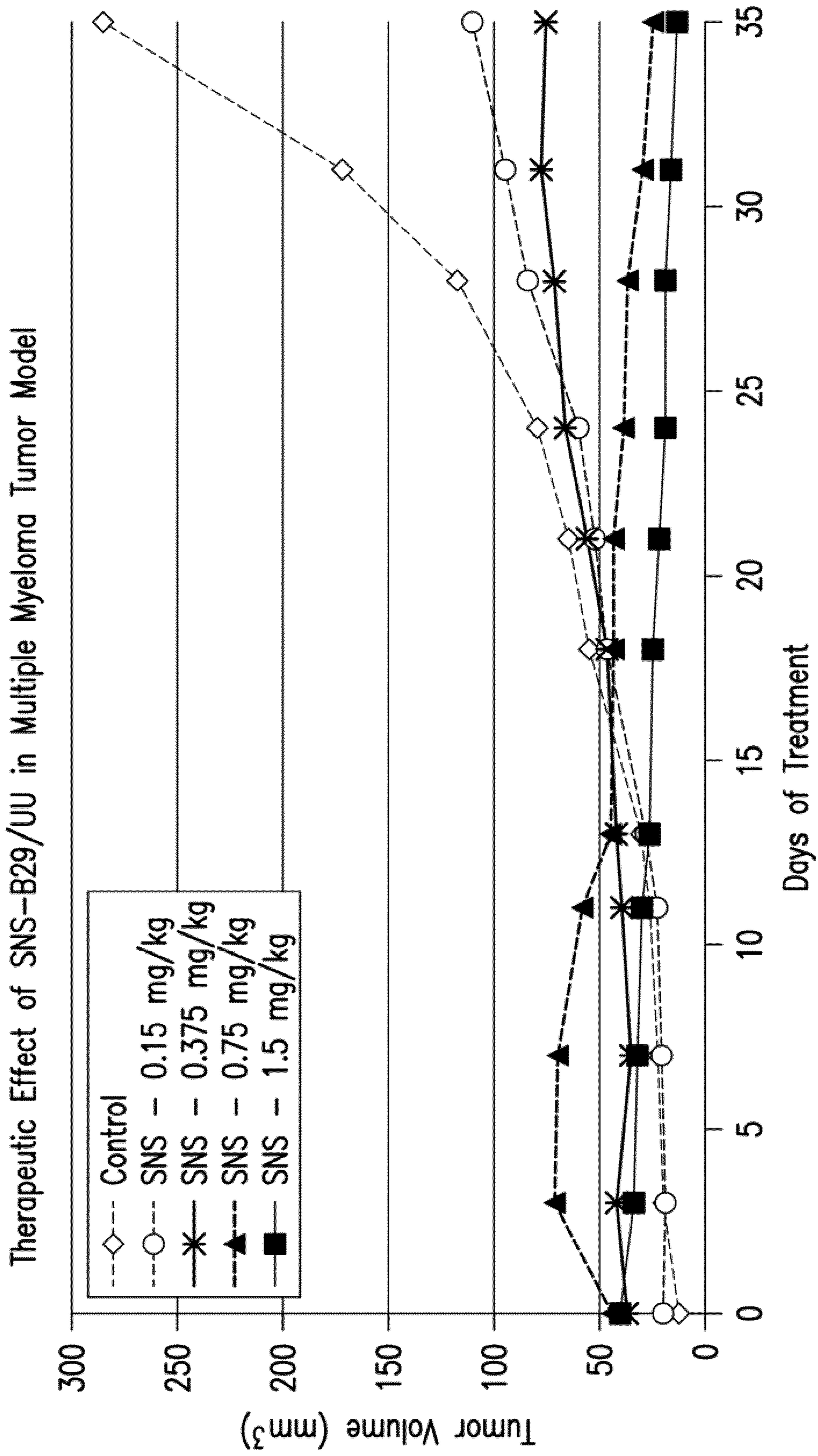


FIG.26

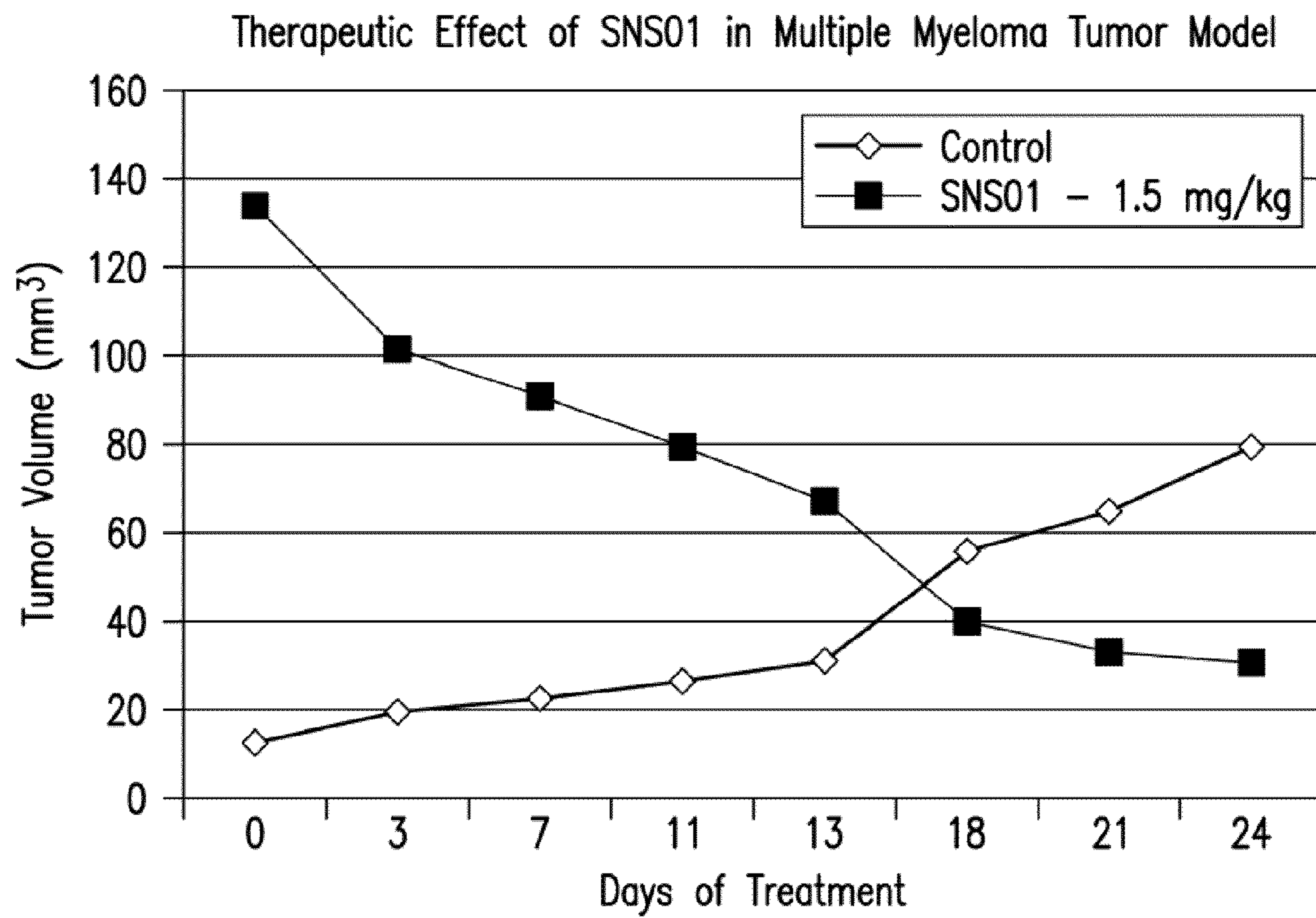


FIG.27

Sequence of eIF5A1<sup>k50R</sup> cDNA

Nucleotide:

Start codon: ATGStop codon: TAAHA-tag: ATGTACCCATACGACGTCCCAGACTACGCT

ATAGGGAGACCCAAGCTGGAGACCATGTACCCATACGACGTCCCAGACTACG  
CTGGAAGCTTAATGGCAGATGATTTGGACTTCGAGACAGGAGATGCAGGGGC  
CTCAGCCACCTTCCCAATGCAGTGCTCAGCATTACGTAAGAATGGTTTTGTG  
GTGCTCAAGGGCCGGCCATGTAAGATCGTCGAGATGTCTACTTCGAAGACTG  
GCAGGCATGGCCATGCCAAGGTCCATCTGGTTGGTATTGATATTTTTACTGG  
GAAGAAATATGAAGATATCTGCCCGTCGACTCATAACATGGATGTCCCCAAC  
ATCAAAAGGAATGATTTCCAGCTGATTGGCATCCAGGATGGGTACCTATCCC  
TGCTCCAGGACAGTGGGGAGGTACGAGAGGACCTTCGTCTGCCTGAGGGAGA  
CCTTGGCAAGGAGATTGAGCAGAAGTATGACTGTGGAGAAGAGATCCTGATC  
ACAGTGCTGTCCGCCATGACAGAGGAGGCAGCTGTTGCAATCAAGGCCATGG  
CAAATAACTGGAATTC

Protein:

HA

MYPYDVDPDYAGSLMADDLDFETGDAGASATFPMQCSALRKNFVVLKGRPCK  
IVEMSTSKTGRHGHAKVHLVGIDIFTGKKYEDICPSTHNMDVPNIKRNDFQL  
IGIQDGYLSLLQDSGEVREDLRLPEGDLGKEIEQKYDCGEEILITVLSAMTE  
EAAVAIKAMAK

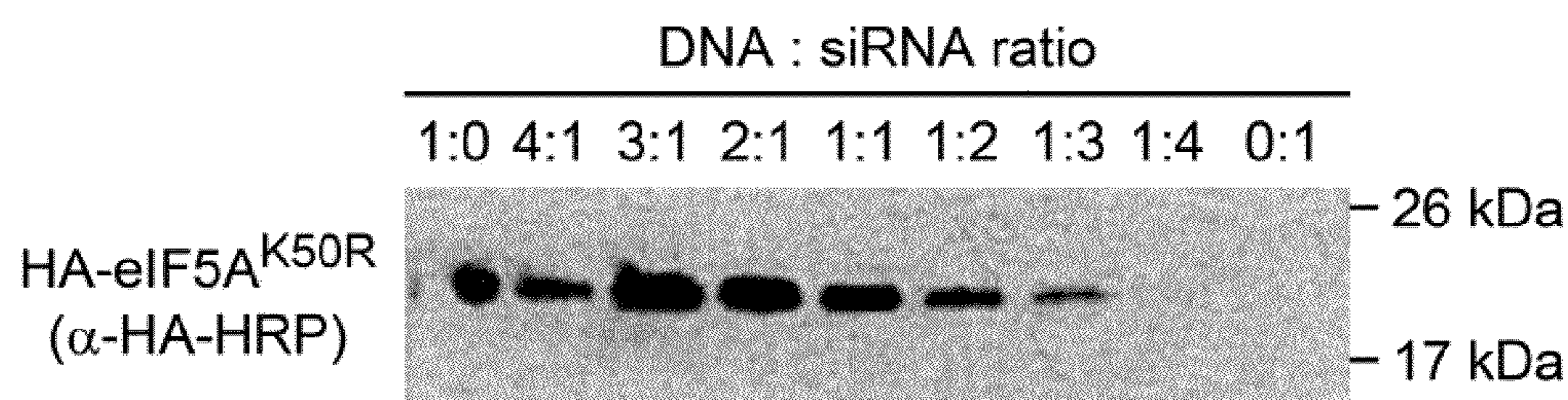
FIG. 28



Alignment between human eIF5A1 and human eIF5A1<sup>k50R</sup>

1					50
eIF5A1	MADDLDFETG	DAGASATFPM	QCSALRKNGF	VVLKGRPCKI	VEMSTSKTGK
K50R	MADDLDFETG	DAGASATFPM	QCSALRKNGF	VVLKGRPCKI	VEMSTSKTGR
Consensus	MADDLDFETG	DAGASATFPM	QCSALRKNGF	VVLKGRPCKI	VEMSTSKTGr
51					100
eIF5A1	HGHAKVHLVG	IDIFTGKKYE	DICPSTHNMD	VPNIKRNDFQ	LIGIQDGYLS
K50R	HGHAKVHLVG	IDIFTGKKYE	DICPSTHNMD	VPNIKRNDFQ	LIGIQDGYLS
Consensus	HGHAKVHLVG	IDIFTGKKYE	DICPSTHNMD	VPNIKRNDFQ	LIGIQDGYLS
101					150
eIF5A1	LLQDSGEVRE	DLRLPEGDLG	KEIEQKYDCG	EEILITVLSA	MTEEA AVAIK
K50R	LLQDSGEVRE	DLRLPEGDLG	KEIEQKYDCG	EEILITVLSA	MTEEA AVAIK
Consensus	LLQDSGEVRE	DLRLPEGDLG	KEIEQKYDCG	EEILITVLSA	MTEEA AVAIK
151					
eIF5A1	AMAK				

FIG.29



PEI Nanoparticles (N/P = 6)

- 1:0 = 3 μg B29-HA-eIF5A<sup>K50R</sup>
- 4:1 = 2.4 μg B29-HA-eIF5A<sup>K50R</sup> + 0.6 μg h5A1
- 3:1 = 2.25 μg B29-HA-eIF5A<sup>K50R</sup> + 0.75 μg h5A1
- 2:1 = 2 μg B29-HA-eIF5A<sup>K50R</sup> + 1 μg h5A1
- 1:1 = 1.5 μg B29-HA-eIF5A<sup>K50R</sup> + 1.5 μg h5A1
- 1:2 = 1 μg B29-HA-eIF5A<sup>K50R</sup> + 2 μg h5A1
- 1:3 = 0.75 μg B29-HA-eIF5A<sup>K50R</sup> + 2.25 μg h5A1
- 1:4 = 0.6 μg B29-HA-eIF5A<sup>K50R</sup> + 2.4 μg h5A1
- 0:1 = 3 μg h5A1

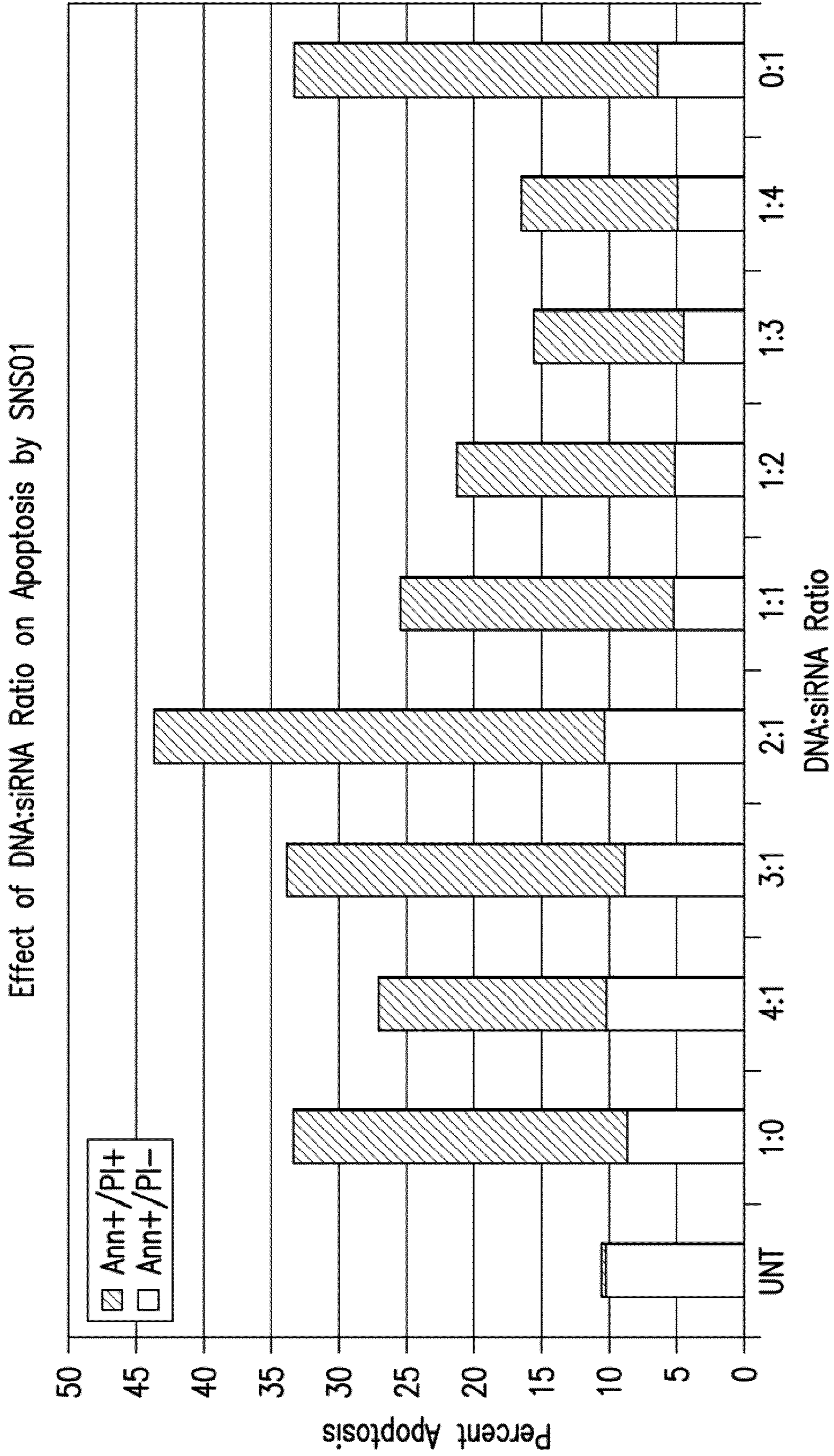
B29-HA-eIF5A<sup>K50R</sup> : plasmid DNA  
B-cell specific promoter/enhancer  
(B29) in pCpG vector (same vector  
as pExp5A) expressing HA-tagged  
eIF5A<sup>K50R</sup>

h5A1 : siRNA targeting human  
eIF5A (GLP-grade)

PEI : GLP-grade

Effect of DNA:siRNA ratio on HA-eIF5A<sup>K50R</sup> Expression

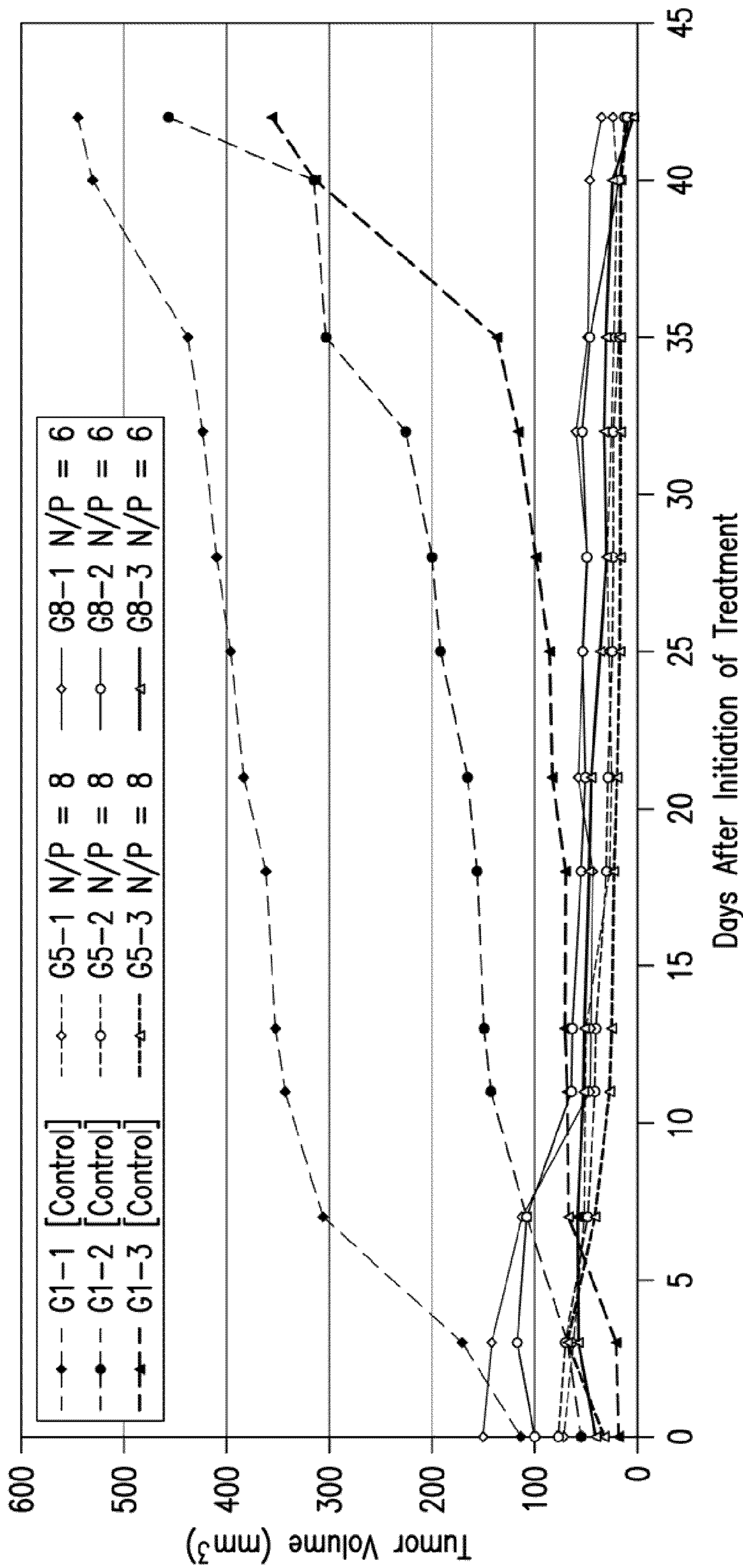
**FIG. 30**



Apoptosis Assay: Effect of DNA:siRNA ratio on apoptosis induced by nanoparticle transfection.

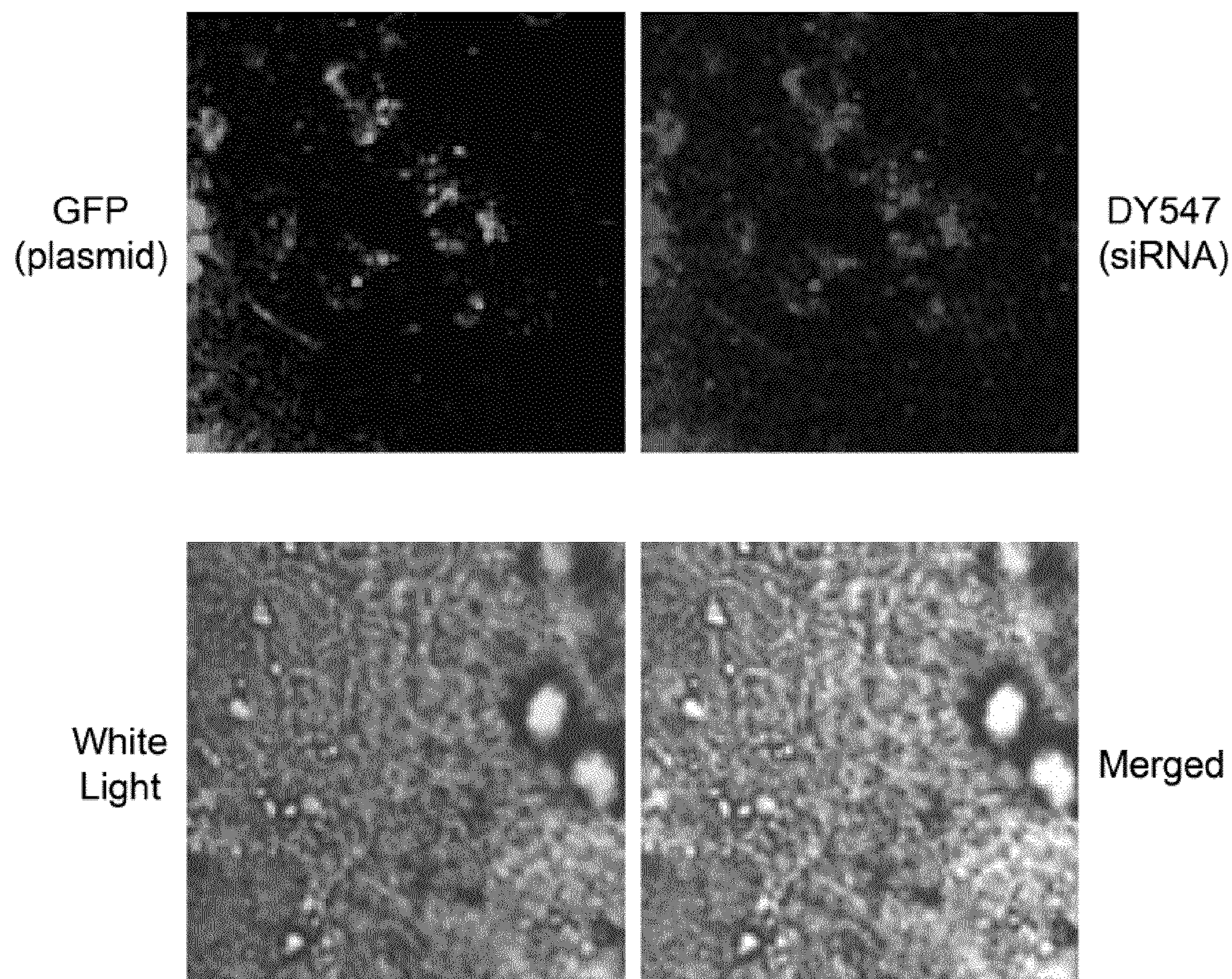
FIG.31

Individual Tumor Volumes of KAS Tumor-Bearing Mice Treated with PEI Complexes at an N/P ratio of Either 6 or 8



Administration of PEI complexes (N/P = 6 or 8) containing eIF5A1<sup>K50R</sup> plasmid and eIF5A1 siRNA (siSTABLE or non-siSTABLE) inhibits growth of multiple myeloma subcutaneous tumors and results in tumor shrinkage.

FIG.32



Tumour section 48 hours after injection with nanoparticles containing pExp-GFP (GFP under control of B-cell-specific promoter) and DY547-siRNA (fluorescently-labelled siRNA). Co-localized expression of GFP and DY547 is observed in tumour section following confocal microscopy indicating that the nanoparticles are being effectively taken up by tumour tissue and that nanoparticles are delivering plasmid and siRNA to the same cell.

**FIG.33**

## COMPOSITIONS COMPRISING SIRNA AND PLASMIDS

This application claims priority to U.S. provisional application 61/034,830, which was filed on Mar. 8, 2008, and which is herein incorporated by reference in its entirety.

### FIELD OF THE INVENTION

The present invention relates to the combinatorial use of siRNA targeted against a gene to knock down or knock out expression of the endogenous gene, and use of a polynucleotide encoding a exogenous gene to affect expression of that gene.

### BACKGROUND OF THE INVENTION

Relatively recently, researchers observed that double stranded RNA (“dsRNA”) could be used to inhibit protein expression. This ability to silence a gene has broad potential for treating human diseases, and many researchers and commercial entities are currently investing considerable resources in developing therapies based on this technology.

Double stranded RNA induced gene silencing can occur on at least three different levels: (i) transcription inactivation, which refers to RNA guided DNA or histone methylation; (ii) siRNA induced mRNA degradation; and (iii) mRNA induced transcriptional attenuation.

It is generally considered that the major mechanism of RNA induced silencing (RNA interference, or RNAi) in mammalian cells is mRNA degradation. Initial attempts to use RNAi in mammalian cells focused on the use of long strands of dsRNA. However, these attempts to induce RNAi met with limited success, due in part to the induction of the interferon response, which results in a general, as opposed to a target-specific, inhibition of protein synthesis. Thus, long dsRNA is not a viable option for RNAi in mammalian systems.

More recently it has been shown that when short (18-30 bp) RNA duplexes are introduced into mammalian cells in culture, sequence-specific inhibition of target mRNA can be realized without inducing an interferon response. Certain of these short dsRNAs, referred to as small inhibitory RNAs (“siRNAs”), can act catalytically at sub-molar concentrations to cleave greater than 95% of the target mRNA in the cell. A description of the mechanisms for siRNA activity, as well as some of its applications are described in Provost et al. (2002) Ribonuclease Activity and RNA Binding of Recombinant Human Dicer, *EMBO J.* 21(21): 5864-5874; Tabara et al. (2002) The dsRNA Binding Protein RDE-4 Interacts with RDE-1, DCR-1 and a DexH-box Helicase to Direct RNAi in *C. elegans*, *Cell* 109(7):861-71; Ketting et al. (2002) Dicer Functions in RNA Interference and in Synthesis of Small RNA Involved in Developmental Timing in *C. elegans*; Martinez et al., Single-Stranded Antisense siRNAs Guide Target RNA Cleavage in RNAi, *Cell* 110(5):563; Hutvagner & Zamore (2002) A microRNA in a multiple-turnover RNAi enzyme complex, *Science* 297:2056.

From a mechanistic perspective, introduction of long double stranded RNA into plants and invertebrate cells is broken down into siRNA by a Type III endonuclease known as Dicer. Sharp, RNA interference—2001, *Genes Dev.* 2001, 15:485. Dicer, a ribonuclease-III-like enzyme, processes the dsRNA into 19-23 base pair short interfering RNAs with characteristic two base 3' overhangs. Bernstein, Caudy, Hammond, & Hannon (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference, *Nature* 409:363. The

siRNAs are then incorporated into an RNA-induced silencing complex (RISC) where one or more helicases unwind the siRNA duplex, enabling the complementary antisense strand to guide target recognition. Nykanen, Haley, & Zamore (2001) ATP requirements and small interfering RNA structure in the RNA interference pathway, *Cell* 107:309. Upon binding to the appropriate target mRNA, one or more endonucleases within the RISC cleaves the target to induce silencing. Elbashir, Lendeckel, & Tuschl (2001) RNA interference is mediated by 21- and 22-nucleotide RNAs, *Genes Dev.* 15:188, FIG. 1.

The interference effect can be long lasting and may be detectable after many cell divisions. Moreover, RNAi exhibits sequence specificity. Kisielow, M. et al. (2002) Isoform-specific knockdown and expression of adaptor protein ShcA using small interfering RNA, *J. Biochem.* 363:1-5. Thus, the RNAi machinery can specifically knock down one type of transcript, while not affecting closely related mRNA. These properties make siRNA a potentially valuable tool for inhibiting gene expression and studying gene function and drug target validation. Moreover, siRNAs are potentially useful as therapeutic agents against: (1) diseases that are caused by over-expression or misexpression of genes; and (2) diseases brought about by expression of genes that contain mutations.

Successful siRNA-dependent gene silencing depends on a number of factors. One of the most contentious issues in RNAi is the question of the necessity of siRNA design, i.e., considering the sequence of the siRNA used. Early work in *C. elegans* and plants circumvented the issue of design by introducing long dsRNA (see, for instance, Fire, A. et al. (1998) *Nature* 391:806-811). In this primitive organism, long dsRNA molecules are cleaved into siRNA by Dicer, thus generating a diverse population of duplexes that can potentially cover the entire transcript. While some fraction of these molecules are non-functional (i.e., induce little or no silencing) one or more have the potential to be highly functional, thereby silencing the gene of interest and alleviating the need for siRNA design. Unfortunately, due to the interferon response, this same approach is unavailable for mammalian systems. While this effect can be circumvented by bypassing the Dicer cleavage step and directly introducing siRNA, this tactic carries with it the risk that the chosen siRNA sequence may be non-functional or semi-functional.

A number of researches have expressed the view that siRNA design is not a crucial element of RNAi. On the other hand, others in the field have begun to explore the possibility that RNAi can be made more efficient by paying attention to the design of the siRNA.

To treat various diseases or disorders, the upregulation of certain proteins is desirable but this may not be all that is needed. For example, the combinatorial use of siRNA to knock down or knock out expression of an endogenous protein or a different protein may be needed. The present invention fulfills this need and provides methods of treating cancer, especially multiple myeloma.

Cancer, including multiple myeloma are diseases which would benefit from the ability to induce apoptosis. Conventional therapies for of multiple myeloma include chemotherapy, stem cell transplantation, high-dose chemotherapy with stem cell transplantation, and salvage therapy. Chemotherapies include treatment with Thalomid®(thalidomide), bortezomib, Aredia® (pamidronate), steroids, and Zometa® (zoledronic acid). However many chemotherapy drugs are toxic to actively dividing non-cancerous cells, such as of the bone marrow, the lining of the stomach and intestines, and the

hair follicles. Therefore, chemotherapy may result in a decrease in blood cell counts, nausea, vomiting, diarrhea, and loss of hair.

Conventional chemotherapy, or standard-dose chemotherapy, is typically the primary or initial treatment for patients with of multiple myeloma. Patients also may receive chemotherapy in preparation for high-dose chemotherapy and stem cell transplant. Induction therapy (conventional chemotherapy prior to a stem cell transplant) can be used to reduce the tumor burden prior to transplant. Certain chemotherapy drugs are more suitable for induction therapy than others, because they are less toxic to bone marrow cells and result in a greater yield of stem cells from the bone marrow. Examples of chemotherapy drugs suitable for induction therapy include dexamethasone, thalidomide/dexamethasone, VAD (vincristine, Adriamycin® (doxorubicin), and dexamethasone in combination), and DVd (pegylated liposomal doxorubicin (Doxil®, Caelyx®), vincristine, and reduced schedule dexamethasone in combination).

The standard treatment for of multiple myeloma is melphalan in combination with prednisone (a corticosteroid drug), achieving a response rate of 50%. Unfortunately, melphalan is an alkylating agent and is less suitable for induction therapy. Corticosteroids (especially dexamethasone) are sometimes used alone for multiple myeloma therapy, especially in older patients and those who cannot tolerate chemotherapy. Dexamethasone is also used in induction therapy, alone or in combination with other agents. VAD is the most commonly used induction therapy, but DVd has recently been shown to be effective in induction therapy. Bortezomib has been approved recently for the treatment of multiple myeloma, but it is very toxic. However, none of the existing therapies offer a significant potential for a cure. Thus, there still remains a need to find a suitable treatment for cancer and multiple myeloma. The present invention fulfills this need.

### SUMMARY OF INVENTION

The present invention relates to the combinatorial use of an siRNA targeted against an endogenous gene to knock out or knock down expression of the endogenous gene in a host and a delivery of a polynucleotide encoding the gene in a delivery vehicle/expression vector to the host to provide expression in the host of the protein encoded by the polynucleotide. A polynucleotide encoding a normal (non faulty) protein (or the protein itself) is administered to the host and is expressed (in the case of the polynucleotide) so that the normal protein can perform its necessary function. The siRNA is preferably designed to target a region of the gene so it either knocks down or knocks out endogenous expression of the faulty protein but at the same time will not effect exogenous expression of the administered polynucleotide encoding the normal protein.

The invention provides a composition comprising a complex of an eIF5A1 siRNA targeted against the 3' end of eIF5A1, an expression vector comprising a polynucleotide encoding a mutant eIF5A1 wherein the mutant eIF5A1 is unable to be hypusinated, and wherein the siRNA and the expression vector are complexed to polyethylenimine to form a complex.

The invention provides a composition comprising an siRNA targeted against a target gene to suppress endogenous expression of the target gene in a subject, and a polynucleotide encoding a target protein capable of being expressed in the subject. In certain embodiments the polynucleotide is in RNAi resistant plasmid (will not be suppressed by the

siRNA). The siRNA and the plasmid are preferably complexed to polyethylenimine to form a complex.

In certain embodiments the siRNA has the sequence shown in FIG. 25 and wherein the polynucleotide encoding the mutant eIF5A1 is eIF5A1<sup>K50R</sup>. The expression vector comprises the a polynucleotide encoding a mutant eIF5A1 and a promoter operably linked to provide expression of the polynucleotide in a subject. The promoter preferably is either tissue specific or systemic. For example, if the composition is used to treat cancer, then preferably the promoter is tissue specific for the tissue in which the cancer resides. For example, for treating multiple myeloma, it is preferable to use a B cell specific promoter, such as B29. In certain embodiments, the expression vector comprises a pCpG plasmid.

In certain embodiments, the eIF5A1 siRNA and the expression vector comprising the mutant eIF5A1 polynucleotide are independently complexed to polyethylenimine, such as in vivo JetPEI™. In other embodiments, the eIF5A1 siRNA and the expression vector comprising the mutant eIF5A1 polynucleotide are complexed together to polyethylenimine.

The present invention further provides a composition comprising an eIF5A1 siRNA targeted against the 3' end of eIF5A1 and an expression vector comprising a polynucleotide encoding a mutant eIF5A1 wherein the mutant eIF5A1 is unable to be hypusinated, and wherein the siRNA and the expression vector are delivered to a subject to treat cancer. The cancer may be any cancer including multiple myeloma.

The present invention further provides a method of treating cancer comprising administering composition of the present invention to a subject (including but not limited to mammals and humans).

The composition may be administered any acceptable route, such as, but not limited to intravenously, intra peritoneally, subcutaneously or intra tumorally. The siRNA and the expression vector may be administered at different times and via different routes or may be administered together at the same time and via the same route. For example, but not limited to, the siRNA may be delivered naked or complexed to a carrier such as in vivo jetPEI via IV and the expression vector may be administered intra tumorally, or both the siRNA and the expression vector may be administered IV or intratumorally, etc.

The present invention provides a method of inhibiting cancer cell growth and/or killing cancer cells. The present invention also provides a method of inhibiting or slowing down the ability of a cancer cell to metastasize. Inhibiting cancer growth includes a reduction in the size of a tumor, a decrease in the growth of the tumor, and can also encompass a complete remission of the tumor. The cancer can be any cancer or tumor, including but not limited to colon cancer, colorectal adenocarcinoma, bladder carcinoma, cervical adenocarcinoma, and lung carcinoma. Preferably the cancer is multiple myeloma.

In preferred embodiments, the eIF-5A is a mutant that is unable to be hypusinated. Exemplary mutants are described herein.

In addition to providing eIF-5A or a polynucleotide encoding eIF-5A to a subject (to provide expression of the eIF-5A), siRNA is provided to knock out or knock down endogenous expression of eIF-5A.

The present invention also provides the use of eIF5A, polynucleotides encoding eIF5A1 and siRNA against eIF5A1 to make a medicament to treat acner kill multiple myeloma cells in a subject having multiple myeloma. Preferably the polynucleotides encoding a mutant eIF-5A are unable to be hypusinated.

The present invention also provides a method of treating sickle cell anemia. A polynucleotide encoding a healthy hemoglobin gene (such as HBB) is administered to a patient suffering from sickle cell anemia. In conjunction, the patient is also administered siRNA that targets the gene encoding the faulty hemoglobin gene (such as the gene encoding the mutant HbS) to knock down or knock out expression of the faulty protein. The treatment may further comprise administration of other known medicines or treatments commonly used in treating sickle cell anemia.

#### BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 provides the amino acid sequence of human eIF-5A1 (SEQ ID NO: 19) and shows various important sites. PHM6-eIF5A1 and Consensus sequence each disclosed as SEQ ID NO: 29.

FIG. 2 shows that mutation of eIF-5A1 at K50 and K67 increases accumulation of transfected protein. See example 1.

FIG. 3 shows that mutation of eIF5A1 at K47, K50 and K67 increases accumulation of transfected protein. See example 2.

FIG. 4 shows that mutation of eIF5A1 at K50 and K67 results in induction of apoptosis when transfected into KAS cells. See example 3.

FIG. 5 shows that mutation of eIF5A1 at K50 and K67 results in induction of apoptosis when transfected into KAS cells. See example 4.

FIG. 6 shows that mutation of eIF5A1 at K50 and K67 results in induction of apoptosis when transfected into KAS cells. See example 5.

FIG. 7A shows transfection with siRNA and treating with an adenovirus that is modified to express eIF-5A1 results in apoptosis in KAS cells. See example 6A. FIG. 7B shows that pre-treatment with eIF5A1 siRNA (against target #1 (SEQ ID NO: 1))(the sequence of the siRNA construct shown in FIG. 25 reduced expression of endogenous eIF5A1 but allows accumulation of RNAi-resistant eIF5A1<sup>K50A</sup> expressed by adenovirus. See example 6B. FIG. 7C shows that pre-treatment with eIF5A1 siRNA against target #1 prior to adenovirus infection reduces expression of phosphorylated NF- $\kappa$ B in human multiple myeloma cells. See example 6C. FIG. 7D shows that pre-treatment with eIF5A1 siRNA against target #1 prior to Adenovirus infection reduces expression of phosphorylated NF- $\kappa$ B and ICAM-1 in human multiple myeloma cells. See example 6D. FIG. 7E shows that siRNA-mediated suppression of eIF5A in human multiple myeloma cells inhibits LPS-mediated induction of NF $\kappa$ B DNA-Binding Activity. The inhibition of NF $\kappa$ B activity by eIF5A siRNA could explain its ability to increase apoptosis induction when combined with over-expression of eIF5A<sup>K50R</sup> since NF- $\kappa$ B regulates many pro-survival and anti-apoptosis pathways. FIG. 7F shows that pretreatment of KAS cells with siRNA increases apoptosis by eIF5A1<sup>K50R</sup> gene delivery in the presence of IL-6. See example 6E.

FIG. 8 shows that co-administration of eIF5A1 plasmid and eIF5A1 siRNA delays growth of multiple myeloma subcutaneous tumours. The data shown is the tumor volume for all the mice in each group. See example 7.

FIG. 9 shows that co-administration of eIF5A1 plasmid and eIF5A1 siRNA delays growth of multiple myeloma subcutaneous tumours. The data shown is the average tumor volume per group  $\pm$  standard error. See example 7.

FIG. 10 shows that co-administration of eIF5A1 plasmid and eIF5A1 siRNA reduces weight of multiple myeloma subcutaneous tumours. See example 7.

FIG. 11 shows that co-administration of eIF5A1 plasmid and eIF5A1 siRNA delays growth of multiple myeloma subcutaneous tumours and results in tumour shrinkage. See Example 8.

FIG. 12 shows that administration of eIF5A1 siRNA intravenously (i.v.) and PEI/eIF5A1K50R plasmid complexes intra-tumourally (i.t.) results in tumour shrinkage of multiple myeloma subcutaneous tumours. See example 9.

FIG. 13A shows that treatment with eIF5A1 plasmid and eIF5A1 siRNA delays growth of multiple myeloma subcutaneous tumours and results in tumour shrinkage. FIG. 13B shows that co-administration of eIF5A1 plasmid and eIF5A1 siRNA results in tumour shrinkage. FIG. 13C shows that administration of eIF5A1 siRNA intravenously (i.v.) and PEI/eIF5A1<sup>K50R</sup> plasmid complexes intra-tumourally (i.t.) results in tumour shrinkage of multiple myeloma subcutaneous tumours.

FIG. 14 shows that intra-venous co-administration of eIF5A1 plasmid and eIF5A1 siRNA delays growth of multiple myeloma subcutaneous tumours. See example 10.

FIG. 15 shows that administration of eIF5A1 siRNA intravenously (i.v.) and PEI/eIF5A1K50R plasmid complexes intra-venously (i.v.) or intra-peritoneal (i.p.) delays growth of multiple myeloma subcutaneous tumours. See example 11.

FIG. 16 shows that treatment with eIF5A1 plasmid and eIF5A1 siRNA delays growth of multiple myeloma subcutaneous tumours.

FIG. 17 shows that co-administration of eIF5A1 plasmid and eIF5A1 siRNA delays growth of multiple myeloma subcutaneous tumours and results in tumour shrinkage. See example 12.

FIG. 18 shows that administration of eIF5A1 siRNA intravenously (i.v.) and PEI/eIF5A1K50R plasmid complexes intra-tumourally (i.t.) results in tumour shrinkage of multiple myeloma subcutaneous tumours. See example 13.

FIG. 19 shows co-administration of eIF5A1<sup>K50R</sup> plasmid, driven by either the EF1 or B29 promoter, and eIF5A1 siRNA delays growth of multiple myeloma subcutaneous tumours and results in tumour shrinkage (KAS-SQ-5). See example 14.

FIG. 20 shows co-administration of eIF5A1 siRNA increases anti-tumor effect of eIF5A1<sup>K50R</sup> plasmid, driven by either the EF1 or B29 promoter, on multiple myeloma subcutaneous tumours and results in reduced tumor burden (KAS-SQ-5). See example 15.

FIG. 21 shows eIF5A1 siRNA synergistically increases apoptosis resulting from infection with Ad-eIF5A in lung adenocarcinoma cells. See example 16.

FIG. 22 shows the map of pExp5A, the construction of which is described in Example 17.

FIG. 23 shows the predicted sequence of pExp5A (3371 bp (SEQ ID NO: 30)). See example 17.

FIG. 24 shows the reexpression of eIF5A1<sup>K50R</sup> in various cell lines. See example 18.

FIG. 25 shows the target sequence (SEQ ID NO: 31) and the sequence of a preferred eIF5A1 siRNA (SEQ ID NOS 31 and 32, respectively, in order of appearance).

FIG. 26 provides the results of efficacy studies in multiple myeloma. See example 21.

FIG. 27 provides the results of efficacy studies in multiple myeloma. See example 21.

FIG. 28 provides the sequence of eIF-5A1<sup>K50R</sup> cDNA (SEQ ID NO: 33) and protein (SEQ ID NO: 34). FIG. 28 also discloses the HA-tag as residues 25-54 of SEQ ID NO: 33.

FIG. 29 provides the alignment of human eIF-5A1 (SEQ ID NO: 19) against human eIF5A1<sup>K50R</sup> (SEQ ID NO: 6). The Consensus sequence is disclosed as SEQ ID NO: 6.



FIG. 30 shows the effect of DNA:siRNA ratio on HA-eIF5A<sup>K50R</sup> expression. See example 23.

FIG. 31 shows the effect of DNA:siRNA ratio on apoptosis induced by nanoparticle transfection. See example 24.

FIG. 32 shows administration of PEI complexes (N/P=6 or 8) containing eIF5A1K50R plasmid and eIF5A1 siRNA (siSTABLE or non-siSTABLE) inhibits growth of multiple myeloma subcutaneous tumours and results in tumour shrinkage. See example 25.

FIG. 33 shows that the JET PEI™ nanoparticles are being effectively taken up by tumour tissue and that nanoparticles are delivering plasmid and siRNA to the same cell. See example 26.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the combinatorial use of an siRNA targeted against an endogenous gene to knock out or knock down expression of the endogenous gene in a subject and a of a polynucleotide encoding the gene in a delivery vehicle/expression vector provided to the subject to provide expression in the host of the protein encoded by the polynucleotide.

This combination is useful in treating a subject with a disease or condition caused by the existence of a faulty or mutant protein, i.e. where the protein produced in the subject is unable to perform its necessary function or alternatively, fowls up a metabolic pathway or biomolecule interaction because of its faulty structure. The siRNA is designed to target the gene encoding the faulty protein, and knock down or knock out expression of that faulty protein. A polynucleotide encoding a normal (non faulty) protein is administered to the subject and is expressed in the subject so that the normal protein is available to perform its necessary function.

In another embodiment, instead of administering the polynucleotide encoding the desired protein, the protein is administered to the subject. The terms protein, peptide and polypeptide are used herein interchangeably.

The siRNA is preferably designed to target a certain region of the gene so it either knocks down or knocks out endogenous expression of the faulty protein but at the same time will not effect exogenous expression of the administered polynucleotide encoding the normal protein. For example, the siRNA may target the 3'UTR so it does not effect exogenous expression of the administered sense construct (the polynucleotide encoding the protein). By knocking down or knocking out endogenous expression of the faulty gene, there will be less or none of the faulty protein to compete with the normal protein expressed from the exogenous polynucleotide.

One example of a disease state where this application would be useful concerns sickle cell anemia. Sickle cell anemia is a blood disorder that affects hemoglobin, the protein found in red blood cells (RBCs) that helps carry oxygen throughout the body.

Sickle cell anemia occurs when a person inherits two abnormal genes (one from each parent) that results in expression of a mutant hemoglobin (Hbs). The mutant hemoglobin causes the RBCs to change shape. Red blood cells with normal hemoglobin (hemoglobin A, or HbA) move easily through the bloodstream, delivering oxygen to all of the cells of the body. They can easily "squeeze" through even very small blood vessels. Sickle cell anemia occurs because the abnormal form of hemoglobin (HbS) tends to clump together, making red blood cells sticky, stiff, and more fragile, and causing them to form into a curved, sickle shape.

Although several hundred HBB gene variants are known, sickle cell anemia is most commonly caused by the hemoglobin variant HbS. In this variant, the hydrophobic amino acid valine takes the place of hydrophilic glutamic acid at the sixth amino acid position of the HBB polypeptide chain. This substitution creates a hydrophobic spot on the outside of the protein structure that sticks to the hydrophobic region of an adjacent hemoglobin molecule's beta chain. This clumping together (polymerization) of HbS molecules into rigid fibers causes the "sickling" of red blood cells.

Polymerization occurs only after red blood cells have released the oxygen molecules that they carry to various tissues throughout the body. Once red blood cells return to the lungs where hemoglobin can bind oxygen, the long fibers of HbS molecules depolymerize or break apart into single molecules. Cycling between polymerization and depolymerization causes red blood cell membranes to become rigid. The rigidity of these red blood cells and their distorted shape when they are not carrying oxygen can result in blockage of small blood vessels. This blockage can cause episodes of pain and can damage organs.

Sickle cell anemia is an autosomal recessive genetic disorder. For the disease to be expressed, a person must inherit either two copies of HbS variant or one copy of HbS and one copy of another variant. Carriers, who have one copy of the normal HBB gene (HbA) and one copy of HbS, are described as having sickle cell trait and do not express disease symptoms.

Thus, one embodiment of the present invention provides a method of treating subjects with sickle cell anemia. siRNA targeted to the HBB gene is administered to the patient. The siRNA is designed to knock down and preferably knock out the expression of the Hbs variant of hemoglobin. A polynucleotide encoding a normal hemoglobin is provided to the subject so the subject expresses a normal hemoglobin. The siRNA is also designed so that it will not interfere with expression of the exogenous polynucleotides encoding the normal hemoglobin. Thus, the subject no longer makes the variant hemoglobin (or makes substantially less) and instead makes normal healthy hemoglobin, resulting in more normal red blood cells, which function normally.

The present invention is also useful in situations where a post translational modification occurs to the protein, which causes or leads to a disease state. siRNA is used to knock down expression of the endogenous protein so none or less is available for the post translational modification. Then, a polynucleotide encoding a protein is provided to the patient for exogenous expression. The protein is modified so that it is unable to be post translationally modified. This protein is then available to the body for its appropriate use, but will not lead to the disease state because it is not able to be post translationally modified. One skilled in the art would understand different post translational modifications. For example, after translation, the posttranslational modification of amino acids extends the range of functions of the protein by attaching to it other biochemical functional groups such as acetate, phosphate, various lipids and carbohydrates, by changing the chemical nature of an amino acid (e.g. citrullination) or by making structural changes, like the formation of disulfide bridges. Also, enzymes may remove amino acids from the amino end of the protein, or cut the peptide chain in the middle. For instance, the peptide hormone insulin is cut twice after disulfide bonds are formed, and a propeptide is removed from the middle of the chain; the resulting protein consists of two polypeptide chains connected by disulfide bonds. Also, most nascent polypeptides start with the amino acid methionine because the "start" codon on mRNA also codes for this

amino acid. This amino acid is usually taken off during post-translational modification. Other modifications, like phosphorylation, are part of common mechanisms for controlling the behavior of a protein, for instance activating or inactivating an enzyme. Another post translational modification includes the hypusination of eukarotic initiation factor 5A (eIF5A) by deoxyhypusine synthase (DHS).

Thus, the invention provides a method of altering expression of a gene in a subject, wherein a polynucleotide encoding a protein is provided to a patient and is expressed in the patient. The protein may be a normal/wild type protein or a mutated protein. Expression of the corresponding endogenous gene is suppressed with the siRNA that is administered to the subject.

The method further comprises providing a construct comprising a polynucleotide encoding the target protein wherein the polynucleotide is expressed in the subject to produce the target protein. In certain embodiments where the endogenous gene expresses a faulty protein, the polynucleotide is designed to encode either a normal/healthy protein. The siRNA is administered to suppress expression of the faulty endogenous protein. In certain embodiments where the endogenous gene expresses a normal healthy protein, the polynucleotide is designed to encode a mutant protein that can not be postrationally modified as would occur with a normal/healthy or non mutant protein. The siRNA is administered to suppress expression of the endogenous protein so there is less of this protein to be available for posttranslational modification.

In certain embodiments, the siRNA is chosen or designed to target regions of the gene so as to not effect expression of the exogenous polynucleotide. For example, the siRNA may target the 3' UTR or 3' end.

The siRNA may be delivered to the patient as either naked siRNA or naked siRNA stabilized for serum. The siRNA may be either injected systemically, i.e. IP or IV. Alternatively, the siRNA may be injected or delivered locally to the desired area of the body. In certain embodiments, the siRNA may be administered in a delivery vehicle such as but not limited to dendrimers, liposomes, or polymers.

The polynucleotides encoding the desired protein may be administered through any delivery means that provide or allow expression of the nucleotide. The term polynucleotide and nucleotide are used herein interchangeably. Delivery may be through any viral or non-viral mechanism, such as but not limited to plasmids, expression vectors, viral constructs, adenovirus constructs, dendrimers, liposomes, or polymers.

In certain embodiments an expression plasmid having reduced CpG dinucleotides is used to express the polynucleotides. Any promoter capable of promoting expression of the polynucleotide may be used, which may be chosen based on the application desired for the therapy. For example, for killing multiple myeloma, a promoter specific for B cells may be desirable, such as human B29 promoter/enhancer. In other embodiments, the promoter may be another tissue specific promoter, or may be a system promoter.

The polynucleotides encoding the target protein may be delivered through IV or subcutaneous injection or any other biologically suitable delivery mechanism.

Alternatively, the polynucleotides may be delivered in liposomes or any other suitable "carrier" or "vehicle" that provides for delivery of the DNA (or plasmid or expression vector) to the target tumor or cancer cells. See for example, Luo, Dan, et al., *Nature Biotechnology*, Vol. 18, January 2000, pp. 33-37 for a review of synthetic DNA delivery systems. Thus, it may be preferable to deliver the nucleotides/plasmid/expression vector via a vehicle of nanometer size

such as liposomes, dendrimers or a similar non-toxic nanoparticle. The vehicle preferably protects the nucleotides/plasmid/expression vector from premature clearance or from causing an immune response while delivering an effective amount of the nucleotides/plasmid/expression vector to the subject, tumor or cancer cells. Exemplary vehicles may range from a simple nano-particle associated with the nucleotides/plasmid/expression vector to a more complex pegylated vehicle such as a pegylated liposome having a ligand attached to its surface to target a specific cell receptor.

Liposomes and pegylated liposomes are known in the art. In conventional liposomes, the molecules to be delivered (i.e. small drugs, proteins, nucleotides or plasmids) are contained within the central cavity of the liposome. One skilled in the art would appreciate that there are also "stealth," targeted, and cationic liposomes useful for molecule delivery. See for example, Hortobagyi, Gabriel N., et al., *J. Clinical Oncology*, Vol. 19, Issue 14 (July) 2001:3422-3433 and Yu, Wei, et al., *Nucleic Acids Research*. 2004, 32(5);e48. Liposomes can be injected intravenously and can be modified to render their surface more hydrophilic (by adding polyethylene glycol ("pegylated") to the bilayer, which increases their circulation time in the bloodstream. These are known as "stealth" liposomes and are especially useful as carriers for hydrophilic (water soluble) anticancer drugs such as doxorubicin and mitoxantrone. To further the specific binding properties of a drug carrying liposome to a target cell, such as a tumor cell, specific molecules such as antibodies, proteins, peptides, etc. may be attached on the liposome surface. For example, antibodies to receptors present on cancer cells maybe used to target the liposome to the cancer cell. In the case of targeting multiple myeloma, folate, II-6 or transferring for example, may be used to target the liposomes to multiple myeloma cells.

Dendrimers are also known in the art and provide a preferable delivery vehicle. See for example Marjoros, Istvan, J., et al, "PAMAM Dendrimer-Based Multifunctional Conjugate for Cancer Therapy: Synthesis, Characterization, and Functionality," *Biomacromolecules*, Vol. 7, No. 2, 2006; 572-579, and Majoros, Istvan J., et al., *J. Med. Chem*, 2005. 48, 5892-5899 for a discussion of dendrimers.

In a preferred embodiment, the delivery vehicle comprises a polyethylenimine nanoparticle. An exemplary polyethylenimine nanoparticle is the *in vivo*-jetPEI™, currently produced by Polyplus Transfection, Inc. *In vivo*-jetPEI™ is cationic polymer transfection agent useful as a DNA and siRNA delivery agent. *In vivo*-jetPEI™ from Polyplus Transfection is a linear polyethylenimine reagent that provides reliable nucleic acid delivery in animals. It is used for gene therapy (Ohana et al., 2004. *Gene Ther Mol Bio* 8:181-192; Vernejoul et al., 2002. *Cancer Research* 62:6124-31), RNA interference, (Urbain-Klein et al., 2004. *Gene therapy* 23:1-6; Grelinski et al., 2006. *Human Gene Therapy* 17:751-66), and genetic vaccination (Garzon et al., 2005. *Vaccine* 23:1384-92). *In vivo* JET-PEI is currently in use in human clinical trials as a delivery vector for cancer gene therapy (Lemkine et al., 2002. *Mol. Cell. Neurosci.* 19:165-174).

*In vivo*-jetPEI™ condenses nucleic acids into roughly 50 nm nanoparticles, which are stable for several hours. As a result of this unique protection mechanism, aggregation of blood cells following injection is reduced compared to other reagents thereby preventing restricted diffusion within a tissue, erythrocyte aggregation and microembolia. These nanoparticles are sufficiently small to diffuse into the tissues and enter the cells by endocytosis. *In vivo*-jetPEI™ favors nucleic acids release from the endosome and transfer across of the nuclear membrane.

In a preferred embodiment, both the siRNA and a vector/plasmid comprising the polynucleotide are administered to the subject via an *in vivo*-jetPEI™ complex. The siRNA and the vector/plasmid comprising the polynucleotide maybe complexed together via a polymer complex such as polyethylenimine or the *in vivo* jetPEI™ complex or may separately complexed to a polymer. For instance, where the siRNA and the vector/plasmid comprising the polynucleotide are to be administered separately to the subject (separately in the meaning of time and/or delivery site) it is preferable to have the siRNA and the polynucleotide complexed to a different carrier. Where the administration will be at the same time and at the same site, it may be preferable to complex the siRNA and the polynucleotide together.

In another embodiment, instead of a plasmid or vector being administered to deliver a polynucleotide that will be expressed in the subject, the protein *per se* is delivered to the subject. The protein may be either isolated or may be synthetic.

One embodiment of the present invention provides a method of treating cancer in a subject, including mammals and humans. Treating cancer includes, but is not limited to inducing apoptosis in cancer cells, killing cancer cells, reducing the number of cancer cells and reducing tumor volume/weight. The method comprises administering a composition comprising eIF5A1 siRNA and a polynucleotide encoding a mutant eIF5A1. The composition and eIF5A1 siRNA and a polynucleotide encoding a mutant eIF5A1 are discussed herein below.

All cells produce eukaryotic initiation factor 5A (“eIF-5A”) (or also referred to herein as “factor 5A”). Mammalian cells produce two isoforms of eIF-5A1 (eIF-5A1 and eIF-5A2). eIF-5A1 has been referred to as apoptosis-specific eIF-5A, as it is upregulated in cells undergoing apoptosis. Human eIF-5A1 has the accession number NM 001970 and is shown in FIG. 1. It is believed that eIF-5A1 is responsible for shuttling out of the nucleus subsets of mRNAs encoding proteins necessary for apoptosis. eIF-5A2 has been referred to as proliferation eIF-5A as it is believed to be responsible for shuttling out of the nucleus subsets of mRNAs encoding proteins necessary for cellular proliferation. See Liu & Tartakoff (1997) Supplement to Molecular Biology of the Cell, 8, 426a. Abstract No. 2476, 37th American Society for Cell Biology Annual Meeting, and Rosorius et al. (1999) J. Cell Science, 112, 2369-2380.

Both factor 5As are post translationally modified by deoxyhypusine synthase (“DHS”). DHS hypusinates the eIF-5As. Hypusine, a unique amino acid, is found in all examined eukaryotes and archaeobacteria, but not in eubacteria, and eIF-5A is the only known hypusine-containing protein. Park (1988) J. Biol. Chem., 263, 7447-7449; Schumann & Klink (1989) System. Appl. Microbiol., 11, 103-107; Bartig et al. (1990) System. Appl. Microbiol., 13, 112-116; Gordon et al. (1987a) J. Biol. Chem., 262, 16585-16589. Hypusinated eIF-5A is formed in two post-translational steps: the first step is the formation of a deoxyhypusine residue by the transfer of the 4-aminobutyl moiety of spermidine to the  $\alpha$ -amino group of a specific lysine of the precursor eIF-5A catalyzed by deoxyhypusine synthase. The second step involves the hydroxylation of this 4-aminobutyl moiety by deoxyhypusine hydroxylase to form hypusine.

The amino acid sequence of eIF-5A is well conserved between species, and there is strict conservation of the amino acid sequence surrounding the hypusine residue in eIF-5A, which suggests that this modification may be important for survival. Park et al. (1993) Biofactors, 4, 95-104. This assumption is further supported by the observation that inac-

tivation of both isoforms of eIF-5A found to date in yeast, or inactivation of the DHS gene, which catalyzes the first step in their activation, blocks cell division. Schnier et al. (1991) Mol. Cell. Biol., 11, 3105-3114; Sasaki et al. (1996) FEBS Lett., 384, 151-154; Park et al. (1998) J. Biol. Chem., 273, 1677-1683. However, depletion of eIF-5A protein in yeast resulted in only a small decrease in total protein synthesis suggesting that eIF-5A may be required for the translation of specific subsets of mRNA's rather than for protein global synthesis. Kang et al. (1993), “Effect of initiation factor eIF-5A depletion on cell proliferation and protein synthesis,” in Tuite, M. (ed.), Protein Synthesis and Targeting in Yeast, NATO Series H. The recent finding that ligands binding eIF-5A share highly conserved motifs also supports the importance of eIF-5A. Xu & Chen (2001) J. Biol. Chem., 276, 2555-2561. In addition, the hypusine residue of modified eIF-5A was found to be essential for sequence-specific binding to RNA, and binding did not provide protection from ribonucleases.

The present inventors have shown that when polynucleotides encoding eIF-5A are administered to cells, there is an increase in apoptosis those cells. They have shown that they have been able to push cancer cells into apoptosis by administering eIF-5A1 polynucleotides that are then expressed in the cancer cells. See co-pending application Ser. Nos. 10/200, 148; 11/287,460; 11/293,391 and 11/637,835, all of which are incorporated by reference in their entirety.

The present inventors have additionally determined that when cells have a build up of the hypusinated form of factor 5A, the cells enter into a survival mode and do not undergo apoptosis as they normally would over time. Notably, in cancer cells, there is a significant amount of hypusinated factor 5A and thus, the cells do not enter into apoptosis (and do not die). Thus, to treat cancer by killing the cancer cells (push the cancer cells to enter into the apoptosis pathway), a polynucleotide encoding eIF-5A1 is administered to the subject or to the cancer cells or tumor to provide increased expression of eIF-5A1, which in turn causes apoptosis in the cancer cells and ultimately cell death and tumor shrinkage. However, if one were to only provide polynucleotides encoding the eIF-5A1 protein to up regulate gene expression of eIF-5A1 and not also use siRNA to knock down endogenous expression of eIF-5A1, there is a tug of war: the eIF-5A1 expression directing the cells towards the apoptosis pathway competes with the presence of the hypusinated factor 5A directing the cells towards the cell survival pathway. The present invention eliminates this tug of war and represents an improvement over only increasing expression of eIF-5A1. The polynucleotides administered to the subject or cell are mutated so that the resulting expressed protein can not be hypusinated. In addition endogenous expression of factor 5A is knocked out/down with siRNA targeted against eIF-5A so there is none/less endogenous eIF-5A1 around to be hypusinated. Thus, since there is no (or substantially less) hypusinated eIF-5A in the cells, they are not pushed into survival mode.

The polynucleotide encoding a mutated eIF-5A1 is preferably mutated so that it can not be hypusinated and thus will not be available to drive the cell into survival mode. For example, in one embodiment, the polynucleotide encoding eIF-5A is mutated to so that the lysine (K) at position 50, which is normally hypusinated by DHS, is changed to an alanine (A) (which can not be hypusinated). This mutant is denoted as K50A.

In another embodiment, the lysine at position 67 is changed to an arginine (R). This mutant is denoted as (K67R). In another embodiment the lysine (K) at position 67 is changed to an alanine (A) and is denoted as (K67A). In another

embodiment, the lysine (K) at position 50 is changed to an arginine (K50R) and another embodiment provides a mutant where the lysine (K) at position 47 is changed to an arginine (K47R).

In other embodiments, a double mutant is used. One double mutant is where the lysine (K) at position 50 is changed to an arginine (R) and the lysine (K) at position 67 is changed to an arginine (R). This double mutant is referred to as K50R/K67R. This double mutant is similarly unable to be hypusinated but the changes in the amino acids do not alter the 3-D structure of eIF-5A1 as much as the single mutation (K50A). The double mutation thus provides a protein that is very similar in 3-D shape and folding as the wild type and thus is more stable than the single mutant. Being more stable, it exists longer in the body to provide longer therapeutic benefit. Thus, the body will have the factor 5A it needs for normal cell function but it will not be able to hypusinated so the cells do not get locked into the cell survival mode and escape apoptosis.

Another double mutant is where the lysine (K) at position 47 is changed to an arginine (R) and the lysine at position 50 is changed to an arginine (R). This mutant is denoted as (K47R/K50R). The invention provides another double mutant where the lysine (L) at position 50 is changes to an alanine (A) and the lysine at position 67 is changes to an alanine (A). This mutant is denoted as (K50A/K67A).

Because the body needs factor 5A for normal cell survival and healthy cell proliferation, it is preferable not to shut off expression completely in the subject with the siRNA, if the siRNA is delivered systemically. Control of eIF-5A expression can be achieved by either using an siRNA that is not as good at shutting off expression (i.e. shuts down or reduces expression but does not completely shut off expression) or alternatively, or utilizing a dosing and/or treatment regimen to balance expression levels to allow normal growth and functioning of healthy cells but also to push cancerous cells to apoptosis.

Alternatively, one may utilize local delivery of siRNA. If the siRNA is delivered locally to the cancer cell or tumor, then the expression is preferably knocked out. By knocking out expression, there is no factor 5A around that can be hypusinated and thus there is no hypusinated eIF-5A to lock the cells into survival mode. Since the siRNA is delivered locally to the cancer or tumor, there is no need to have eIF-5A available for regular cell growth.

In certain embodiments, the endogenous gene is eIF5A1. siRNA targeted against eIF5A1 is administered to the subject to suppress expression of the endogenous eIF-5A1. In certain embodiments the siRNA comprises SEQ ID NO:1 or SEQ ID NO:2 or is any siRNA targeted against eIF5A1 that will suppress expression of endogenous eIF-5A1. In certain embodiments, the eIF5A1 is human eIF-5A1 (shown in FIG. 1) and the subject is a human. Other siRNAs targeted against human eIF-5A1 are known and disclosed in co-pending application Ser. Nos. 11/134,445; 11/287,460; 11/184,982; 11/293,391; 11/725,520; 11/725,470; 11/637,835. In other embodiments, the subject is a mammal and the eIF5A1 is specific to the mammal. For example, the subject is a dog and the eIF5A1 is canine eIF5A1. In certain embodiments, the siRNA consists essentially of the siRNA construct shown in FIG. 25. For example, the siRNA contains nucleic acids targeted against the eIF5A1 but also contains overhangs such as U or T nucleic acids or also contains tags, such as a his tag (often referred to as HA tag—which is often used in in vivo studies). Molecules or additional nucleic acids attached at either the 5' or 3' end (or even within the consecutive string of nucleic acids shown in FIG. 25, for example) may be included

and fall within the “consisting essentially of” as long as the siRNA construct is able to reduce expression of the target gene. Preferably the siRNA targets regions of the eIF5A1 gene so as to not effect expression of the exogenous polynucleotide. For example the eIF5A1 siRNA targets the 3' UTR or the 3' end. The siRNA shown in FIG. 25 an exemplary eIF5A1 siRNA.

The polynucleotide encodes eIF5A1 wherein the polynucleotide is mutated to encode an eIF5A1 variant. The mutated eIF5A1 is designed so that the variant eIF5A1 can not be post translationally modified (can not be hypusinated). Exemplary mutants are discussed herein above.

In the case of cancer involving solid tumors, it may be desirable to deliver the siRNA directly to the tumor. The siRNA maybe administered separately with respect to time as well as the delivery site from the polynucleotide or may administered together at the same time and/or at the same delivery site. One skilled in the art would understand that the timing of administration of the siRNA may be necessarily administered when the endogenous protein is being translated and not after it is already made.

Although the present inventors have earlier shown that eIF5A1 is non toxic to normal tissue (see pending application Ser. No. 11/293,391, filed Nov. 28, 2005, which is incorporated herein by reference in its entirety), a delivery complex (as compared to direct administration of the eIF5A polynucleotides/plasmid/expression vector) may be preferred. A preferred delivery system provides an effective amount of eIF5A1 to the subject, tumor or group of cancer cells, as well as preferably provides a targeted delivery to the tumor or group of cancer cells. Thus, in certain embodiments, it is preferable to deliver the eIF5A1 nucleotides/plasmid/expression vector via a vehicle of nanometer size such as liposomes, dendrimers or a similar non-toxic nano-particle such as a polyethylenimine polymer (such as an in vivo JetPEI™ complex).

The eIF5A1 protein may also be delivered directly to the site of the tumor. One skilled in the art would be able to determine the dose and length of treatment regimen for delivery of eIF5A1 protein.

The molecular basis for the induction of apoptosis by eIF5A1 is discussed below.

#### Death Receptor Signaling

Treatment of cancer cells with Ad-eIF5A1 (adenovirus with a wild type eIF5A1) or Ad-eIF5A1(K50A) induces activation of caspase 8, which is initiated by death receptor—ligand binding, and caspase 3, the executioner caspase. These are likely to be indirect effects of eIF5A1, and the fact that caspase 8 and caspase 3 are also activated following treatment with eIF5A1(K50A), which cannot be hypusinated, indicates that the effect is attributable to lysine<sub>50</sub> eIF5A1. Treatment with Ad-eIF5A1 also appears to result in up-regulation of death receptors as shown previously with upregulation of TNFR1.

#### Mitochondrial Pathway

Direct or indirect involvement of lysine<sub>50</sub> eIF5A1 in the mitochondrial pathway for apoptosis is supported by a number of observations including the finding that caspase 9 is activated by treatment of cancer cells with either eIF5A1 or eIF5A1(K50A). As well, p53, which plays a role in activation of the mitochondrial apoptotic pathway, appears to be regulated by eIF5A1. For example, treatment of cancer cells with Actinomycin D up-regulates p53, and this up-regulation of p53 is inhibited by eIF5A1 siRNA. Consistent with this, treatment of cancer cells with Ad-eIF5A1 up-regulates p53 mRNA. Treatment of cancer cells with eIF5A1 also induces migration of Bax from the cytosol to mitochondria, ensuing

loss of mitochondrial membrane potential and release of cytochrome C from the intramitochondrial space into the cytosol. In addition, this treatment results in up-regulation of cleaved Bcl2, Bim and spliced Bim, which are all pro-apoptotic.

#### MAPK Signaling

In addition, the present inventors have obtained evidence for the involvement of eIF5A1 in MAPK signaling related to apoptosis. For example, treatment of cancer cells with Ad-eIF5A1 up-regulated P-JNK, which in turn inhibits anti-apoptotic Bcl2. In addition Ad-eIF5A1 and Ad-eIF5A1 (K50A) both induce the formation of P-p38, which can in turn initiate apoptosis by impacting a variety of pro-apoptotic agents including TNFR1 & TNF; FAS & FASL; caspase 8; Bid; Cytochrome C and Caspase 3.

#### NF- $\kappa$ B Signaling

There is evidence that NF- $\kappa$ B signaling supports myeloma growth. For example, myeloma cell adhesion to bone marrow stromal cells induces NF- $\kappa$ B-dependent transcriptional up-regulation of IL-6, which is both a growth and anti-apoptotic factor in multiple myeloma [Chauhan et al. (1996) *Blood* 87, 1104.] In addition, TNF- $\alpha$  secreted by myeloma cells activates NF- $\kappa$ B in bone marrow stromal cells, thereby up-regulating IL-6 transcription and secretion. TNF- $\alpha$  also activates NF- $\kappa$ B in myeloma cells resulting in up-regulation of the intracellular adhesion molecule-1 (ICAM-1; CD54) and the vascular cell adhesion molecule-1 (VCAM-1; CD106) on both myeloma cells and bone marrow stromal cells [Hideshima et al. (2001) *Oncogene* 20, 4519]. This in turn enhances the association of myeloma cells with bone marrow stromal cells [Hideshima et al. (2001) *Oncogene* 20, 4519]. Conversely, these effects are inhibited by blocking TNF- $\alpha$ -induced NF- $\kappa$ B activation [Hideshima et al. (2001) *Oncogene* 20, 4519]. Indeed, it seems likely that NF- $\kappa$ B mediates protection against TNF- $\alpha$ -induced apoptosis in myeloma cells [Hideshima et al. (2002) *JBC* 277, 16639]. These and other observations have prompted the view that NF- $\kappa$ B signaling may be an attractive target for multiple myeloma therapies.

The inventors have shown that eIF5A1 siRNA inhibits both the activation of NF- $\kappa$ B and the formation of ICAM-1 in human myeloma cells. These observations indicate that eIF5A1 plays a role in NF- $\kappa$ B activation, and inasmuch as the ensuing effects of NF- $\kappa$ B activation are pro-survival in nature, we predict that this activation is mediated, directly or indirectly, by hypusinated eIF5A1.

#### IL-1

Over-production of the pro-inflammatory cytokine, IL-1, by myeloma cells is a characteristic feature of multiple myeloma that leads to deterioration of bone tissue. eIF5A1 siRNA has been shown to dramatically reduce the overproduction of IL-1 induced by an LPS challenge in mice.

One embodiment of the present invention provides a method of treating multiple myeloma. Multiple myeloma ("MM") is a progressive and fatal disease characterized by the expansion of malignant plasma cells in the bone marrow and by the presence of osteolytic lesions. Multiple myeloma is an incurable but treatable cancer of the plasma cell. Plasma cells are an important part of the immune system, producing immunoglobulins (antibodies) that help fight infection and disease. Multiple myeloma is characterized by excessive numbers of abnormal plasma cells in the bone marrow and overproduction of intact monoclonal immunoglobulins (IgG, IgA, IgD, or IgE; "M-proteins") or Bence-Jones protein (free monoclonal light chains). Hypocalcaemia, anemia, renal damage, increased susceptibility to bacterial infection, and impaired production of normal immunoglobulin are common clinical manifestations of multiple myeloma. Multiple

myeloma is often also characterized by diffuse osteoporosis, usually in the pelvis, spine, ribs, and skull.

The present invention seems to be well suited to treat multiple myeloma because of the stimulation feedback loop found in multiple myeloma. For instance, multiple myeloma produces IL-1 in low concentrations in bone marrow. The IL-1 in turn stimulates stromal cells to produce IL-6, which then goes onto stimulate growth of the multiple myeloma. The inventors have previously shown (see pending application Ser. Nos. 11/725,539 and 11/184,982) that siRNA directed against eIF-5A1 was able to inhibit expression of proinflammatory cytokines, such as IL-1; TNF- $\alpha$ , and IL-8). Thus, the siRNA would not only knock down expression of eIF-5A so less is available for hypusination, it would also cut off or decrease the IL-1/IL-6 feedback loop.

An siRNA targeting human eIF5A was used to suppress levels of endogenous hypusinated eIF5A in tumours, while an RNAi-resistant plasmid expressing a mutant of eIF5A (eIF5A<sup>K50R</sup>), that is incapable of being hypusinated, was used to raise the levels of unmodified eIF5A in vivo. Intra-tumoural injection of PEI nanocomplexes containing eIF5A siRNA inhibited MM tumour growth by more than 80% (\*\*p=0.0003) versus complexes containing a control siRNA, indicating that suppressing levels of hypusinated eIF5A has an anti-tumoural effect. PEI complexes containing an eIF5A<sup>K50R</sup> expression plasmid had a similar effect and inhibited tumour growth by more than 70% (\*\*p=0.001) versus complexes containing a control plasmid. Thus, MM tumour growth can be inhibited either by suppression of the growth-promoting hypusinated eIF5A or by increasing levels of the pro-apoptotic unhypusinated form of eIF5A. Intra-tumoural delivery of complexes containing both eIF5A siRNA and RNAi-resistant eIF5A<sup>K50R</sup> plasmid had a synergistic effect on tumour growth and resulted in significant tumour shrinkage, inhibiting tumour growth by 94% (\*\*p=0.0002). Intra-venous delivery of eIF5A siRNA/eIF5A<sup>K50R</sup> PEI complexes also efficiently reduced tumour growth by 95% (\*\*p=0.002) indicating systemic delivery of the therapeutic is feasible.

Both local and systemic delivery of eIF5A siRNA/eIF5A<sup>K50R</sup> pDNA PEI complexes resulted in a significant anti-tumoural response in multiple myeloma.

The present invention further provides a composition useful in the treatment of cancer, including multiple myeloma. In a preferred embodiment, the composition is a complex of a plasmid DNA encoding point-mutated eIF5A1 that cannot be hypusinated and eIF5A1 siRNA that selectively suppresses endogenous human eIF5A1 but has no effect on the point-mutated eIF5A1 encoded by the plasmid. eIF5A1 siRNAs and polynucleotides encoding mutant eIF5A1 are discussed above. The plasmid DNA and the siRNA are both preferably complexed to PEI (polyethylenimine) nanoparticles. They may be complexed separately and administered separately or together or they may be complexed together. The DNA and the RNA bind to positively charged amino groups on the PEI and are released when the nanoparticles are taken up into cells. It has been demonstrated that PEI-nucleic acid complexes are effectively taken up into both dividing and non-dividing cells.

The plasmid DNA preferably encodes eIF5A1(K50R) which, like eIF5A1(K50A), cannot be hypusinated and, accordingly, is strongly apoptogenic. The expression of eIF5A1(K50R) is preferably regulated by a B-cell-specific promoter.

The eIF5A1 siRNA is preferably specific to the 3'-end of endogenous human eIF5A1 and has no effect on expression of the trans eIF5A1(K50R). An exemplary preferred eIF5A1

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siRNA comprises, consists essentially of or consists of the siRNA shown in FIG. 25. The rationale for including the eIF5A1 siRNA is: (1) to deplete endogenous eIF5A1, which is almost all hypusinated and hence in the pro-survival form; (2) to inhibit activation of NF- $\kappa$ B, and thereby reduce the production of IL-6 and the formation of intracellular adhesion molecules; and (3) to inhibit the formation of IL-1. That eIF5A1 siRNA acts synergistically with eIF5A1(K50R) to induce apoptosis in myeloma cells. Inasmuch as (2) and (3) above are pro-survival events, they are likely mediated by hypusinated eIF5A1, and hence not affected by eIF5A1 (K50R) which cannot be hypusinated. This approach results in a larger pool of unhypusinated eIF5A leading to apoptosis of cancer cells, including multiple myeloma cells, with little effect on healthy cells.

A preferred composition is referred to herein as SNS01. SNS01 is a complex containing both, an RNAi-resistant plasmid DNA encoding eIF5A<sup>K50R</sup> driven by a promoter that restricts expression to cells of B-cell origin (including myeloma cells) for enhanced safety, and an siRNA targeting human eIF5A with dTdT 3' overhangs for enhanced nuclease resistance and which the siRNA and the plasmid are complexed to in vivo JetPEI™.

## EXAMPLES

## Example 1

## Transfection of HeLaS3 Cells with Wild Type and Variants of eIF-5A1

HeLa S3 cells were transfected using Lipofectamine 2000 with plasmids expressing HA-tagged eIF5A1 variants including wild-type eIF5A1 (WT), eIF5A1K50R (K50R), eIF5A1K67R (K67R), eIF5A1K67A (K67A), eIF5A1K47R/K50R (K4750R), eIF5A1K50R/K67R (K5067R), or eIF5A1K50A/K67A (K5067A). A plasmid expressing LacZ was used as a control. At 24 and 48 hours (A) or 28 and 52 hours (B) after transfection, the cell lysate was harvested and fractionated by SDS-PAGE. Expression levels of transfected eIF5A1 was detected using an antibody against HA. Result: Mutation of eIF5A1 at a lysine in the putative ubiquination site (K67R) increased the accumulation of the eIF5A1 transgene above wild-type (A). Mutation of eIF5A1 at the lysine required for hypusination (K50R) also increased accumulation of eIF5A1 transgene above wild-type eIF5A1 (B). A double mutant form of eIF5A1 (K50A/K67A) was expressed particularly well when compared to the unmutated wild-type eIF5A1 transgene (A+B). See FIG. 2.

## Example 2

## Transfection of KAS Cells with Wild Type and Variants of eIF-5A1

KAS cells were transfected using PAMAM dendrimer (FMD44) with plasmids expressing HA-tagged eIF5A1 variants including wild-type eIF5A1 (5A1), eIF5A1K67A (K67A), eIF5A1K50A/K67A (K50A1K67A), eIF5A1K50R (K50R), eIF5A1K47R (K47R), eIF5A1K67R (K67R), eIF5A1K47R/K50R (K47R K50R), or eIF5A1K50R/K67R (K50R K67R). A plasmid expressing LacZ was used as a control. 48 hours (after transfection, the cell lysate was harvested and fractionated by SDS-PAGE. Expression levels of transfected eIF5A1 was detected using an antibody against HA. Equal loading was verified using an antibody against actin. Result: Mutation of eIF5A1 at a lysine in the putative

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ubiquination site (K67A or K67R) increased the accumulation of the eIF5A1 transgene above wild-type. Mutation of eIF5A1 at the lysine required for hypusination (K50R) or at an acetylation site (K47R) also increased accumulation of eIF5A1 transgene above wild-type eIF5A. A double mutant form of eIF5A1 (K50A/K67A) was also expressed at higher levels when compared to the unmutated wild-type eIF5A1 transgene. See FIG. 3.

## Example 3

## Transfection of KAS Cells using PAMAM Dendrimer

KAS cells were transfected using PAMAM dendrimer (FMD45-2) with plasmids expressing HA-tagged eIF5A1 variants including eIF5A1K50R (K50R), eIF5A1K50A/K67A (K50A/K67A), or eIF5A1K50R/K67R (K50R K67R). A plasmid expressing LacZ was used as a control. Seventy-two hours after transfection, the cells were stained with Annexin/PI and analyzed by FACS. Cells that stained positively for Annexin V and negatively for PI (propidium iodide) were considered to be in the early stages of apoptosis (Ann+/PI-) and cells that stained positively for both Annexin V and PI were considered to be in the late stages of apoptosis (Ann+/PI+). Result: Mutation of eIF5A1 at a lysine in the hypusination site (K50R) or in the putative ubiquination site (K67R), as well as the double mutant (K50A/K67A) resulted in apoptosis of KAS cells significantly above the levels of the LacZ control. See FIG. 4.

## Example 4

## Transfection of KAS Cells with Plasmids Expressing eIF-5A1 and eIF-5A1 Variants

KAS cells were transfected using Lipofectamine 2000 with plasmids expressing HA-tagged eIF5A1 variants including eIF5A1K50A (K50A), eIF5A1K50R (K50R), eIF5A1K67R (K67R), eIF5A1K50A/K67A (K50A/K67A), or eIF5A1K50R/K67R (K50R K67R). A plasmid expressing LacZ was used as a control. Seventy-two hours after transfection, the cells were stained with Annexin/PI and analyzed by FACS. Cells that stained positively for Annexin V and negatively for PI (propidium iodide) were considered to be in the early stages of apoptosis (Ann+/PI-) and cells that stained positively for both Annexin V and PI were considered to be in the late stages of apoptosis (Ann+/PI+). Result: Mutation of eIF5A1 at a lysine in the hypusination site (K50R) or in the putative ubiquination site (K67R), as well as the double mutant (K50A/K67A) resulted in apoptosis of KAS cells significantly above the levels of the LacZ control. See FIG. 5.

## Example 5

## The use of Mutated eIF-5A1 to Treat KAS Cells Results in Apoptosis

KAS cells were transfected using Lipofectamine 2000 with plasmids expressing HA-tagged eIF5A1 variants eIF5A1K50R (K50R) or eIF5A1K50A/K67A (K50A/K67A). A plasmid expressing LacZ was used as a control. Seventy-two hours aftertransfection, the cells were stained with Annexin/PI and analyzed by FACS. Cells that stained positively for Annexin V and negatively for PI (propidium iodide) were considered to be in the early stages of apoptosis (Ann+/PI-) and cells that stained positively for both Annexin V and

PI were considered to be in the late stages of apoptosis (Ann+/PI+). Result: Mutation of eIF5A1 at a lysine in the hypusination site (K50R) or mutation of eIF5A1 at both the hypusination site and in the putative ubiquitination site (K50A/K67A) resulted in apoptosis of KAS cells significantly above the levels of the LacZ control. See FIG. 6.

#### Example 6A

##### siRNA/Adenovirus-Mediated Killing of Multiple Myeloma Cells

KAS (human multiple myeloma) cells were maintained in S10 media [RPMI 1640 with 4 ng/ml IL-6, 10% fetal bovine serum (FBS), and penicillin/streptomycin (P/S)]. KAS cells were transfected with 58.7 pmoles of siRNA using Lipofectamine 2000 (Invitrogen). Mock transfected cells were treated with Lipofectamine 2000 in the absence of siRNA. Transfection was conducted in the antibiotic-free S10 media.

##### a) siRNAs targeting human eIF5A1:

eIF5A1 siRNA target #1 (the siRNA targets this region of human eIF5A1: 5'-AAGCTGGACTCCTCCTACACA-3' (SEQ ID NO: 1). The siRNA sequence is shown in FIG. 25 and is often referred to herein as h5A1.

eIF5A1 siRNA target #2 eIF5A1 (this siRNA targets this region of human eIF5A1: 5'-AAAGGAATGACTTC-CAGCTGA-3' (SEQ ID NO: 2). (The siRNA sequence is often referred to herein as h5A1-ALT)

b) control siRNA: The control siRNA had the following sequence: sense strand, 5' ACACAUCCUCCUCAG-GUCGdTdT-3' (SEQ ID NO: 3); and antisense strand, 3'-dTdTUGUGUAGGAGGAGUCCAGC-5' (SEQ ID NO: 4).

Other controls that have been used include non-targeting validated siRNAs from Dharmacon since they have been micro-array tested to limit unwanted off-targeting effects. For example, for in vitro work studying NFkB, the control used was Dharmacon's non-targeting siRNA's (sequence D-001700-01) and for in vivo work, the control used was Dharmacon's (sequence D-001810-01).

Four hours after transfection, the cells were pelleted and resuspended in 1 ml of S10 media. Seventy-two hours after the initial siRNA transfection, the transfected KAS cells were counted and seeded at 300,000 cells/well in a 24-well plate and transfected with the same siRNA a second time.

Four hours after transfection, the cells were pelleted and resuspended in 1 ml of S10 media (without IL-6) containing 3000 ifu of either Ad-LacZ (Adenovirus expressing  $\beta$ -galactosidase) or Ad-5A1M (Adenovirus expressing human eIF5A1<sup>K50A</sup>).

Seventy-two hours later the cells were harvested and analyzed for apoptosis by staining with Annexin V-FITC and PI (BD Bioscience) followed by FACS analysis.

a) early apoptosis was defined as cells that were positively stained with Annexin-FITC and negative for PI-staining (Ann+/PI-)

b) total apoptosis was defined as the total of cells either in early apoptosis (Ann+/PI-) or late apoptosis/necrosis (Ann+/PI+)

The 5A1 siRNA targeting #1 targets the 3'UTR of human eIF5A1 and therefore will not affect expression of eIF5A1 from adenovirus. 5A1 siRNA targeting #2 targets within the open reading frame of human eIF5A1 and so it could potentially interfere with expression of eIF5A1 from the adenovirus.

#### Results:

Cells treated with siRNA and infected with adenovirus expressing the eIF-5A1 K50A variant undergo apoptosis in greater numbers than non-treated cells and cells treated only with siRNA. See FIG. 7.

#### Example 6B

##### Pre-Treatment with eIF5A1 siRNA against eIF5A1 Target #1 (Shown in FIG. 25), Reduced Expression of Endogenous eIF5A1 but allows Accumulation of RNAi-Resistant eIF5A1<sup>K50A</sup> Expressed by Adenovirus

KAS cells were transfected using Lipofectamine 2000 with either a control siRNA (C) or one of two siRNAs targeting eIF5A1 (#1 and #2). The eIF5A1 siRNA #1 targets the 3'UTR of eIF5A1 and therefore does not interfere with expression of eIF5A1 from adenovirus since it contains only the open reading frame of eIF5A1. The sequence of the siRNA is shown in FIG. 25. The eIF5A1 #2 siRNA targets the open reading frame of eIF5A1 and will therefore affect expression of both endogenous and exogenously-expressed eIF5A1. Seventy-two hours after the initial transfection hours the cells were transfected with the same siRNA a second time. Four hours later the transfection complexes were removed from the cells and replaced with growth media (-) IL6 containing either Ad-LacZ (L) or Ad-eIF5A1<sup>K50A</sup> (5M). Seventy-two hours later the cell lysate was harvested and analyzed by Western blot using antibodies against eIF5A and actin. See FIG. 7B. Accumulation of virally expressed eIF5A1 can be observed (lane 1 vs lane 2) and reduction of eIF5A expression by eIF5A1 siRNAs targeting #1 and #2 can be clearly seen (lanes 5 and 7 vs lane 3). As expected, the eIF5A1 siRNA #1 does not affect accumulation of the virally expressed eIF5A1<sup>K50A</sup> (lane 6 vs lane 4) while the eIF5A1 siRNA #2 only moderately affects expression of the virally-expressed transgene (lane 8 vs lane 4).

#### Example 6C

##### Pre-Treatment with eIF5A 1 siRNA against Target #1 Prior to Adenovirus Infection Reduces Expression of Phosphorylated NF- $\kappa$ B in Human Multiple Myeloma Cells

KAS cells were transfected using Lipofectamine 2000 with either a control siRNA (hC) or an siRNA targeting eIF5A1 (#1). The eIF5A1 siRNA #1 targets the 3'UTR of eIF5A1 and will therefore not interfere with expression of eIF5A1 from adenovirus since it contains only the open reading frame of eIF5A1. Seventy-two hours after the initial transfection hours the cells were transfected with the same siRNA a second time. Four hours later the transfection complexes were removed from the cells and replaced with growth media (+) IL6 containing either Ad-LacZ (L) or Ad-eIF5A1<sup>K50A</sup> (5M). Twenty-four hours later the cell lysate was harvested and analyzed by Western blot using antibodies against phospho-NF- $\kappa$ B p65 (Ser 536) and eIF5A. As expected, the eIF5A1 siRNA #1 does not affect accumulation of the virally expressed eIF5A1<sup>K50A</sup>. Phosphorylation of NF- $\kappa$ B p65 at serine 536 regulates acti-

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vation, nuclear localization, protein-protein interactions, and transcriptional activity. See FIG. 7C.

## Example 6D

Pre-Treatment with eIF5A1 siRNA #1 Prior to Adenovirus Infection Reduces Expression of Phosphorylated NF-kB and ICAM-1 in Human Multiple Myeloma Cells

KAS cells were transfected using Lipofectamine 2000 with either a control siRNA (C) or one of two siRNAs targeting eIF5A1 (#1 and #2). Seventy-two hours after the initial transfection hours the cells were transfected with the same siRNA a second time. Four hours later the transfection complexes were removed from the cells and replaced with growth media (+) IL6. Twenty-four hours after the second transfection, the cells were stimulated with 40 ng/ml TNF- $\alpha$  and cell lysate was harvested at 0, 4, or 24 hours and analyzed by Western blot using antibodies against phospho-NF-kB p65 (Ser 536), ICAM-1 and actin. A reduction in TNF- $\alpha$  induced NF-kB p65 phosphorylation (ser 536) and ICAM-1 expression was observed following transfection with both eIF5A1-specific siRNAs. Phosphorylation of NF-kB p65 at serine 536 regulates activation, nuclear localization, protein-protein interactions, and transcriptional activity. ICAM-1 is an inter-cellular adhesion surface glycoprotein that is believed to be involved in the pathogenesis of multiple myeloma. See FIG. 7D.

## Example 6E

Pretreatment of KAS Cells with siRNA Increases Apoptosis by eIF5A1<sup>K50R</sup> Gene Delivery in the Presence of IL-6

KAS cells were transfected with either control siRNA (hcon) or human eIF5A1 siRNA (h5A1) using Lipofectamine 2000. Seventy-two hours later the cells were re-transfected with siRNA. PEI complexes of empty vector (mcs) or eIF5A1<sup>K50R</sup> (K50R) plasmids were added to the cells four hours later following removal of siRNA transfection medium. The growth medium used throughout the study contained IL-6. Apoptosis was measured seventy-two hours later by staining the cells with Annexin/PI and FACS analysis. See FIG. 7F.

## Example 7

Co-Administration of eIF-5A1 Plasmid and eIF-5A1 siRNA Delays Growth of Multiple Myeloma Subcutaneous Tumors (FIGS. 8-10)

SCID mice were injected subcutaneously with KAS cells. Treatment was initiated when palpable tumours were observed. Six 3-5 week old SCID/CB17 mice were injected with 10 million KAS-6/1 myeloma cells in 200  $\mu$ L PBS in their right flank and treatment was initiated when the tumours reached a minimum size of 4 mm<sup>3</sup>.

Control mice were injected intra-tumourally 2 times per week with PEI complexes containing pCpG-mcs (empty vector) and control siRNA (control group was made up of 3 mice: C-1, C-2, and C-3). Treated mice were injected intra-tumourally 2 times per week with PEI complexes containing the RNAi-resistant plasmid pCpG-eIF5A1k50R and eIF5A1 siRNA (treated group was made up of 3 mice: 5A-1, 5A-2, and 5A-3). Injections were given at multiple sites within the tumor to prevent reflux and a slow rate of injection was used

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to increase uptake. The data in FIG. 8 shows the tumor volume for all the mice in the group. The data shown in FIG. 9 is the average tumor volume per group  $\pm$  standard error. Asterix denote statistical significance ( $*=p<0.025$ ;  $n=3$ ).

FIG. 10 shows that co-administration of eIF-5A1 plasmid and eIF-5A1 siRNA reduces the weight of multiple myeloma subcutaneous tumors. The data shown is the average tumor weight per group  $\pm$  standard error. Asterix denote statistical significance ( $*=p<0.05$ ;  $n=3$ ).

JET-PEI (PolyPlus) at 2 $\times$ 0.1 ml was used for the in vivo tests. The N/P ratio was 8. The PEI/DNA/siRNA complexes were formed in a total volume of 0.1 ml in 5% glucose. The protocol for forming complexes was as follows.

1. Bring components to room temperature. Keep sterile.
2. Dilute 20  $\mu$ g of plasmid DNA ( $\sim$ 10  $\mu$ l at 2 mg/ml) and 10  $\mu$ g siRNA (10  $\mu$ l at 1 mg/ml) into a total volume of 25  $\mu$ l. Use sterile water to make up difference.
3. Adjust the volume of DNA solution to 50  $\mu$ l 5% glucose by adding 25  $\mu$ l of 10% glucose. Vortex gently and centrifuge briefly.
4. Dilute 4.8  $\mu$ l of in vivo JETPEI into a total volume of 25  $\mu$ l of 10% glucose. Adjust volume to 50  $\mu$ l with sterile water to end up with a final concentration of 5% glucose. Vortex gently and centrifuge briefly.
5. Immediately add 50  $\mu$ l of diluted PEI to the 50  $\mu$ l of diluted DNA (do not reverse the order). Vortex briefly and immediately spin down.
6. Incubate for 15 minutes prior to injection. Complexes are stable for 6 hours.

CpG-free Cloning Vectors and pCpG Plasmids were obtained from InvivoGen. These plasmids are completely devoid of CpG dinucleotides, named pCpG. These plasmids yield high levels of transgene expression both in vitro and in vivo, and in contrast to CMV-based plasmids allow sustained expression in vivo. pCpG plasmids contain elements that either naturally lack CpG dinucleotides, were modified to remove all CpGs, or entirely synthesized such as genes encoding selectable markers or reporters. Synthesis of these new alleles was made possible by the fact that among the sixteen dinucleotides that form the genetic code, CG is the only dinucleotide that is non-essential and can be replaced. Eight codons contain a CG encoding for five different amino acids. All eight codons can be substituted by at least a choice of two codons that code for the same amino acid to create new alleles that code for proteins having amino acid sequences that remain identical to the wild type and thus are as active as their wild-type counterparts. These new alleles are available individually in a plasmid named pMOD from which they can be easily excised.

pCpG plasmids allow long lasting expression in vivo, and represent valuable tools to study the effects of CpGs on gene expression in vivo and in vitro, using cell lines expressing TLR9, as well as their effects on the innate and acquired immune systems.

The empty vector, pCpG-mcs (Invivogen) is a vector with no expressed gene product, only a multiple cloning site, and was used as the control vector. An HA-tagged eIF5A1<sup>K50R</sup> cDNA was subcloned into the NcoI and NheI sites of a pCpG-LacZ vector (Invivogen), from which the LacZ gene had been removed, to give rise to the treatment vector pCpG-eIF5A1 (K50R). The DNA was prepared using Endo-Free Qiagen kit. Endotoxin levels measured and are  $<0.03$  EU/ $\mu$ g; DNA should be at 2 mg/ml in water.

The control siRNA used in the experiments was a microarray validated non-targeting control siRNA from Dharmacon (D-001810-01). The siRNA was obtained with a modification (siSTABLE) to prevent degradation in serum.



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The eIF5A1 siRNA used in the experiments was designed against the 3'UTR of human eIF5A1. There is no similarity between the eIF5A1 siRNA and mouse eIF5A1 and the siRNA should therefore only suppress human (but not mouse) eIF5A1. The siRNA also has no similarity to eIF5A2 (either human or mouse). The siRNA was obtained with a modification (siSTABLE) to prevent degradation in serum. The eIF5A1 siRNA has the following target sequence:

(SEQ ID NO: 5)

5' GCU GGA CUC CUC CUA CAC A (UU) 3

The siSTABLE siRNA was dissolved at 1 mg/ml in water (stored in aliquots at -20 C).

Tumour dimensions of length (l) and width (w) were measured 2-3 times per week using digital calipers. Tumour volume was calculated according to the following equation:

l=length; smallest dimension

w=width; largest dimension

$$\text{tumour volume (mm}^3\text{)}=l^2*w*0.5$$

#### Statistical Analyses

Student's t-test was used for statistical analysis. Significance was deemed to be a confidence level above 95% (p<0.05).

#### Example 8

##### Co-Administration of eIF5A1 Plasmid and eIF5A1 siRNA Delays Growth of Multiple Myeloma Subcutaneous tumors and Results in Tumor Shrinkage

In another study, again SCID mice were injected subcutaneously with KAS cells. Treatment was initiated when palpable tumours were observed. Control mice were injected intra-tumourally 2 times per week with PEI complexes containing pCpG-mcs (empty vector) and control siRNA (control group G-1, G-2 and G-3). Treated mice were injected intra-tumourally 2 times per week with PEI complexes containing the RNAi-resistant plasmid pCpG-eIF5A1k50R (20 µg of plasmid DNA) and eIF5A1 siRNA (10 µg of siRNA)(treated group G-4, G-5 and G-6). The data shown in FIG. 11 is the average tumour volume per group+/-standard error. Asterix denote statistical significance (\*=p<0.025; n=3). Six injections over a period of 21 days were given (red arrows).

#### Example 9

##### Administration of eIF5A1 siRNA Intra-Venously (i.v.) and PEI/eIF5A1K50R Plasmid Complexes Intra-Tumourally (i.t.) Results in Tumour Shrinkage of Multiple Myeloma Subcutaneous Tumours (Group 2B)

SCID mice were injected subcutaneously with KAS cells. When palpable tumours were observed treatment was initiated with an initial tail injection of 50 micrograms of either control siRNA (control group) or human eIF5A1 siRNA (treated group). Control Mice were subsequently treated by intra-tumoural injections 2 times per week with PEI complexes containing pCpG-mcs (empty vector; control group; G-1, G-2, and G-3). Treated mice were subsequently treated by intra-tumoural injections 2 times per week with PEI complexes containing the RNAi-resistant plasmid pCpG-eIF5A1k50R (20 µg plasmid DNA)(treated group; G-4, G-5, and G-6). Control mice continued to receive control siRNA

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(control group R-1, R-2, and R-3) by i.v. injection once per week. Treated mice continued to receive human eIF5A1 siRNA (20 µg)(treated group R-4, R-5, and R-6) by i.v. injection once per week. The data shown in FIG. 12 is the tumor volume for all the mice in each group. Six intramural injections of PEI/DNA (red arrows) and four i.v. injections of siRNA (blue arrows) were given over a period of 21 days.

FIG. 13 provides an overlay of the results from Example 8 and 9. SCID mice were injected subcutaneously with KAS cells. The data shown in FIG. 13 is the average tumor volume for the mice in each group+/-standard error. Asterix denote statistical significance between treated and control groups (\*\*=p<0.01; \*\*\*=p<0.001; n=3).

Protocol for forming PEI complexes:

1. Bring components to room temperature. Keep sterile.
2. Dilute plasmid DNA or plasmid DNA+siRNA into a total volume of 25 µl. Use sterile water to adjust the volume.
  - a) For plasmid DNA only complexes:
    - 20 Dilute 20 µg of plasmid DNA (10 µl at 2 mg/ml) into a total volume of 25 µl. Use sterile water to make up difference.
    - b) For plasmid DNA+siRNA complexes:
      - 25 Dilute 20 µg of plasmid DNA (~10 µl at 2 mg/ml) and 10 µg of siRNA (10 µl at 1 mg/ml) into a total volume of 25 µl. Use sterile water to make up difference.
  3. Adjust the volume of DNA solution to 50 µl 5% glucose by adding 25 µl of 10% glucose (provided with PEI kit). Vortex gently and centrifuge briefly.
  - 30 4. Dilute in vivo JETPEI into a total volume of 25 µl of 10% glucose.
    - a) For plasmid DNA only complexes:
      - 35 Dilute 3.2 µl of in vivo JETPEI into a total volume of 25 µl of 10% glucose. Adjust volume to 50 µl with sterile water to end up with a final concentration of 5% glucose. Vortex gently and centrifuge briefly.
      - b) For plasmid DNA+siRNA complexes:
        - 40 Dilute 4.8 µl of in vivo JETPEI into a total volume of 25 µl of 10% glucose. Adjust volume to 50 µl with sterile water to end up with a final concentration of 5% glucose. Vortex gently and centrifuge briefly.
    5. Immediately add 50 µl of diluted PEI to the 50 µl of diluted DNA (do not reverse the order!). Vortex briefly and immediately spin down.
    - 45 6. Incubate for 15 minutes prior to injection. Complexes are stable for 6 hours.

Regarding the tail-vein injection of siRNA, the initial siRNA injection was 50 micrograms. siRNA was diluted to 0.4 mg/ml in PBS. 125 µl per mouse (50 µg) was injected into the tail vein. Subsequent injections of serum-stabilised siRNA were given two times per week at 20 µg per mouse. siRNA was diluted to 0.4 mg/ml in PBS. 50 µl per mouse (20 µg) was injected into the tail vein.

FIG. 13B shows that co-administration of eIF5A1 plasmid and eIF5A1 siRNA results in tumour shrinkage. SCID mice were injected subcutaneously with KAS cells. Treatment was initiated when palpable tumours were observed. Mice were injected intra-tumourally 2 times per week with PEI complexes containing the RNAi-resistant plasmid pCpG-eIF5A1k50R and eIF5A1 siRNA (treated group; G-4, G-5, and G-6). Six injections over a period of 21 days were given. Forty-two days after the initiation of treatment the mice were sacrificed and the skin under the tumour site was opened and examined for evidence of tumour growth. No tumour growth was observed in any of the group 2A treated mice.

FIG. 13C shows that administration of eIF5A1 siRNA intra-venously (i.v.) and PEI/eIF5A1K50R plasmid com-

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plexes intra-tumourally (i.t.) results in tumour shrinkage of multiple myeloma subcutaneous tumours. SCID mice were injected subcutaneously with KAS cells. When palpable tumours were observed treatment was initiated with an initial injection of 50 micrograms of human eIF5A1 siRNA (treated group). Mice were subsequently treated by intra-tumoural injections 2 times per week with PEI complexes containing the RNAi-resistant plasmid pCpG-eIF5A1k50R (treated group; R-4, R-5, and R-6). Mice continued to receive human eIF5A1 siRNA by i.v. injection once per week. Treatment ended 21 days after initiation of treatment. Forty-two days after the initiation of treatment the mice were sacrificed and the skin under the tumour site was opened and examined for evidence of tumour growth. There was no evidence of tumour growth in one mouse of the treatment group (group 2B).

## Example 10

Intra-Venous Co-Administration of eIF5A1 Plasmid and eIF5A1 siRNA Delays Growth of Multiple Myeloma Subcutaneous Tumours

SCID mice were injected subcutaneously with KAS cells. When palpable tumours were observed treatment was initiated with an initial injection of 50 micrograms of either control siRNA (control group) or human eIF5A1 siRNA (treated group). Mice were subsequently treated by intra-venous (red arrows) or intra-peritoneal injections (green arrow)~twice per week with either PEI complexes containing pCpG-mcs (empty vector; control group A1, A2, and A3) or PEI complexes containing the RNAi-resistant plasmid pCpG-eIF5A1k50R (treated group; A4, A5, and A6). Mice continued to receive either control siRNA (control group A1, A2, and A3) or human eIF5A1 siRNA (treated group A4, A5, and A6) by i.v. injection (blue arrows) once per week. The data shown is the tumour volume for all the mice in each group. The data shown in FIG. 14 is the tumour volume for all the mice in each group.

## Example 11

Administration of eIF5A1 siRNA Intra-Venously (i.v.) and PEI/eIF5A1K50R Plasmid Complexes Intra-Venously (i.v.) or Intra-Peritoneal (i.p.) Delays Growth of Multiple Myeloma Subcutaneous Tumours

SCID mice were injected subcutaneously with KAS cells. When palpable tumours were observed treatment was initiated with an initial injection of 50 micrograms of either control siRNA (control group) or human eIF5A1 siRNA (treated group). Control mice were subsequently treated by intra-venous or intra-peritoneal injections once per week with PEI complexes containing pCpG-mcs (empty vector; control group was three mice: B1, B2, and B3). Treated mice were subsequently treated by intra-venous or intra-peritoneal injections~once per week with PEI complexes containing the RNAi-resistant plasmid pCpG-eIF5A1<sup>K50R</sup> (treated group; B4, B5, and B6). Mice continued to receive either control siRNA (control group B1, B2, and B3) or human eIF5A1 siRNA (treated group was three mice: B4, B5, and B6) by i.v. injection once per week. The experiment began with initial siRNA injection of 50 µg (day-2 on graph in FIG. 15). Subsequent injections used 20 micrograms of siRNA once weekly. The siRNA was given naked, i.e. no delivery vehicle. PEI complexes contained 20 µg of plasmid DNA. The initial

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PEI injection was given i.p. and subsequent injections were given i.v. The data shown in FIG. 15 is the tumor volume for all the mice in each group.

FIG. 16 provides an overlay of Example 10 and 11. SCID mice were injected subcutaneously with KAS cells. Treatment was initiated when palpable tumours were observed. One set of mice received i.v. injections of either control siRNA (control; Group A) or eIF5A1 siRNA (treated; Group A) once per week and either i.v. or i.p. of either PEI complexes containing pCpG-mcs (control; Group A) or PEI complexes containing the RNAi-resistant plasmid pCpG-eIF5A1<sup>K50R</sup> (treated; Group A). A second set of mice were given i.v. or i.p. injections~2 times per week with either PEI complexes containing pCpG-mcs (empty vector) and control siRNA (control; Group B.) or PEI complexes containing the RNAi-resistant plasmid pCpG-eIF5A1k50R and eIF5A1 siRNA (treated; Group B). The data shown is the average tumour volume for the mice in each group+/-standard error. Asterix denote statistical significance between treated and control groups (\*=p<0.05; \*\*\*=p<0.001; n=3).

The protocols for preparing the PEI complexes and the siRNA are as described in previous examples.

## Example 12

Co-Administration of eIF5A1 Plasmid and eIF5A1 siRNA Delays Growth of Multiple Myeloma Subcutaneous Tumours and Results in Tumour Shrinkage

SCID mice were injected subcutaneously with KAS cells. Treatment was initiated when palpable tumours were observed. Control mice were injected intra-tumourally 2 times per week with PEI complexes containing pCpG-mcs (empty vector) and control siRNA (control group had 3 mice: control 1, control 2, and control 3). Treated mice were injected intra-tumourally 2 times per week with PEI complexes containing the RNAi-resistant plasmid pCpG-eIF5A1<sup>K50R</sup> and eIF5A1 siRNA (treated group contained 4 mice: 5A-1, 5A-2, 5A-3, and 5A-4). The intra-tumoural rejections of PEI complexes contained both 20 µg of plasmid DNA and 10 µg of siRNA. The data shown in FIG. 17 is the tumour volume for all the mice in each group.

## Example 13

Administration of eIF5A1 siRNA Intra-Venously (i.v.) and PEI/eIF5A1<sup>K50R</sup> Plasmid Complexes Intra-Tumourally (i.t.) Results in Tumour Shrinkage of Multiple Myeloma Subcutaneous Tumours

SCID mice were injected subcutaneously with KAS cells. When palpable tumours were observed treatment was initiated with an initial injection of 50 micrograms of either control siRNA (control group had three mice: control 1, control 2 and control 3) or human eIF5A1 siRNA (treated group had 3 mice: 5A-1, 5A-2, 5A-3). Control mice were subsequently treated by intra-tumoural injections 2 times per week with PEI complexes containing pCpG-mcs (20 µg) (control group 1-3). Treated mice were subsequently treated by intra-tumoural injections 2 times per week with PEI complexes containing the RNAi-resistant plasmid pCpG-eIF5A1<sup>K50R</sup> (20 µg) (5A-1, 5A-2, 5A-3). Control mice continued to receive either control siRNA (20 µg) by tail vein i.v. injection twice per week. Treated mice continued to receive human eIF5A1 siRNA (20 µg) by tail vein i.v. injection twice per week. The injections were give 48 hours prior to the intra-tumoural

injections. The siRNA was given as naked siRNA, i.e. no delivery vehicle. The data shown in FIG. 18 is the tumour volume for all the mice in each group.

## Example 14

Co-Administration of eIF5A1<sup>K50R</sup> Plasmid, Driven by either the EF1 or B29 Promoter, and eIF5A1 siRNA Delays Growth of Multiple Myeloma Subcutaneous Tumours and Results in Tumour Shrinkage

SCID mice were injected subcutaneously with KAS cells. Treatment was initiated when palpable tumours were observed. Mice were injected intra-tumourally 2 times per week with PEI complexes containing either control vector (G1 and G2) or an eIF5A1 plasmid driven by either the B29 promoter (G3 and G4) or the EF1 promoter (G5 and G6) and either control siRNA (G1, G3, G5) or h5A1 siRNA (G2, G4, G6). The data shown is the average tumour volume $\pm$ standard error for each group. Note: the B29 promoter was intended as a B-cell-specific promoter. However, although the B29 promoter/mCMV enhancer used in this study was found to drive high expression of HA-eIF5A1<sup>K50R</sup> in KAS cells in vitro, it does not appear to be B-cell-specific (likely due to CMV enhancer). See FIG. 19.

## Example 15

Co-Administration of eIF5A1 siRNA Increases Anti-Tumor Effect of eIF5A1<sup>K50R</sup> Plasmid, Driven by either the EF1 or B29 Promoter, on Multiple Myeloma Subcutaneous Tumours and Results in Reduced Tumor Burden

SCID mice were injected subcutaneously with KAS cells. Treatment was initiated when palpable tumours were observed. Mice were injected intra-tumourally 2 times per week with PEI complexes containing either control vector (G1 and G2) or an eIF5A1 plasmid driven by either the B29 promoter (G3 and G4) or the EF1 promoter (G5 and G6) and either control siRNA (G1, G3, G5) or h5A1 siRNA (G2, G4, G6). The mice were sacrificed 24 days after the initiation of treatment and the subcutaneous tumor was removed and weighed. The data shown is the average tumor weight $\pm$ standard error for all groups. See FIG. 20.

## Example 16

eIF5A1 siRNA Synergistically Increases Apoptosis Induction Resulting from Infection with Ad-eIF5A in Lung Adenocarcinoma Cells

A549 cells were infected with either Ad-LacZ or Ad-eIF5A. Cells were transfected with either a control siRNA or an siRNA targeting human eIF5A1 (h5A1) by adding the transfection media to cells immediately following addition of the virus. Four hours after transfection with the siRNA and infection with virus, the media was replaced with fresh media and the cells were incubated for 72 hours prior to labelling with Annexin/PI to detect apoptotic cells. Note: over-expression of eIF5A in this cell line results in the accumulation of unhyposinated eIF5A due to limiting amounts of DHS and DOHH and therefore results in same pro-apoptotic effect as over-expression of eIF5A<sup>K50R</sup>. These data indicate that the synergistic effect in apoptosis caused by simultaneous suppression of hypusinated eIF5A and over-expression

of unhyposinated eIF5A is observed in non-myeloma tumour-types as well. See FIG. 21.

## Example 17

## Construction of Plasmid pExp5A

pExp5A is an expression plasmid with reduced CpG dinucleotides designed to drive expression of human eIF5A1<sup>K50R</sup> predominantly in cells of B cell lineage. The vector is derived from pCpG-LacZ, a plasmid completely devoid of CpG dinucleotides (Invivogen). All the elements required for replication and selection in *E. coli* are free of CpG dinucleotides. The original CMV enhancer/promoter and LacZ gene from the CpG-LacZ vector have been replaced with a human minimal B cell specific promoter (B29/CD79b; Invivogen) and human eIF5A1<sup>K50R</sup>, respectively, in order to drive B-cell specific expression of eIF5A1<sup>K50R</sup>. The B29 DHS4.4 3' enhancer has been introduced into the plasmid downstream of the eIF5A1 expression cassette in order to enhance activity of the B29 promoter and reduce expression in non-B cells (Malone et al. 2006. B29 gene silencing in pituitary cells is regulated by its 3' enhancer. *J. Mol. Biol.* 362: 173-183). Incorporation of the B29 minimal promoter, eIF5A1<sup>K50R</sup>, and the B29 DHS4.4 3' enhancer has introduced 32 CpG dinucleotides into the vector.

Elements for Expression in *E. coli*

Origin of replication: *E. coli* R6K gamma ori.

\* Due to the presence of the R6K gamma origin of replication, pCpG plasmids can only be amplified in *E. coli* mutant strain expressing a pir mutant gene. They will not replicate in standard *E. coli* strains. Therefore, pCpG plasmids are provided with the *E. coli* GT115 strain, a pir mutant also deficient in Dcm methylation (Invivogen).

Bacterial promoter: EM2K, a CpG-free version of the bacterial EM7 promoter.

Selectable marker: Zeocin<sup>TM</sup> resistance gene; a synthetic allele with no CpGs.

Elements for Expression in Mammalian Cells

Mammalian promoter: the human -167 bp minimal B29 (CD79b) promoter for tissue-specific expression in B cells. A synthetic intron (I 140) is also present in the 5'UTR.

Polyadenylation signal: a CpG dinucleotide-free version of the late SV40 polyadenylation signal.

3' Enhancer: the human B29 DHS4.4 3' enhancer.

MAR: Two CpG-free Matrix attached regions (MAR) are present between the bacterial and mammalian transcription units. One MAR is derived from the 5' region of the human IFN- $\beta$  gene and one from the 5' region of the  $\beta$ -globin gene.

The predicted Sequence of pExp5A (3371 bp is provided at FIG. 23.

Amino Acid Sequence of eIF5A1<sup>K50R</sup> (SEQ ID NO: 6)

\* K50R mutation is underlined

Amino Acid Sequence of eIF5A1<sup>K50R</sup>  
\* K50R mutation is underlined

(SEQ ID NO: 6)

MADDLDFETGDAGASATFPMQCSALRKNGFVVLKGRPCKIVEMSTSKTG

RHGHAKVHLVIGIDIFTGKKYEDI CPSTHNMDVPNIKRND FQLIGIQDGY

LSLLQDSGEVREDLRLPEGDLGKEIEQKYDCGEEILITVLSAMTEEA AV  
AIKAMAK

Construction of pExp5A—Outline of Construction:

Step 1: Cloning of B29 DHS4.4 3' enhancer and subcloning into pGEM T easy (Promega)—creates pGEM-4.4enh #8.

Step 2: Subcloning of minimal B29 promoter into pCpG-LacZ (Invivogen)—creates B29-5#3.

Step 3: Subcloning of HA-eIF5A1<sup>K50R</sup> into B29-5#3 vector—creates B29-5#3-eIF5A1<sup>K50R</sup>.

Step 4: Creation of new multiple cloning site in pCpG-mcs (Invivogen)—creates pCpG-Linker4.

Step 5: Subcloning of B29 DS4.4 3' enhancer into pCpG-Linker4—creates pCpG-DHS4.4.

Step 6: Subcloning of B29 promoter+HA-eIF5A1<sup>K50R</sup>+SV40 pA expression cassette into pCpG-DHS4.4 creates pExp-5.

Step 7: Replacement of HA-eIF5A1<sup>K50R</sup> in pExp-5 with non-HA eIF5A1<sup>K50R</sup> creates final vector, pExp5A.

Construction in Detail:

Step 1: Cloning of B29 DHS4.4 3' enhancer and subcloning into pGEM T easy (Promega)—creates pGEM-4.4enh #8.

The B29 DHS4.4 3' enhancer was cloned by PCR from genomic DNA isolated from KAS cells (human multiple myeloma cell line) using the following primers: forward 5'-CAGCAAGGGAGCACCTATG-3' (SEQ ID NO: 7) and reverse 5'-GTTGCAGTGAGCGGAGATG-3' (SEQ ID NO: 8). The primers were designed using the sequence of the human CD79B/GH-1 Intergenic region (Accession AB062674). The resulting 608 bp PCR fragment was subcloned into the pGEM® T easy cloning vector (Promega) and

sequenced. Komatsu et al. 2002. Novel regulatory regions found downstream of the rat B29/Ig-b gene. Eur. J. Biochem. 269: 1227-1236.

5 Sequence of B29 DHS4.4 3' enhancer PCR fragment (297 Bp) in pGEM-4.4enh #8 (SEQ ID NO: 9)

ACCACCCTGGGCCAGGCTGGGCCAAGCCAGGCGGCCCTGTGTTTTCCCC

AGTCTCTGGGCTGCTGGAGGGAACCAGGTTGTTTTGGCATCAGCCTCTAC

10 TGAGCCGGAGCCCTTCTTTTCTGCTGCTTTGCATAGTGGCACTAATTCC

GTCCTCCTACCTCCACCAGGGACCTAGGCAGCCGGGTAGATGGTGGGAGG

AGGCTTCACTTCTCCCCAAGCAGGGTCTCCACCTGCTTGAGGCTGCCCT

15 GGGTTGGGGGAGGCCTTGGCTTTACCTAAAGACTTTTAAACACCTCT

+4.4 regions contains several transcription factor binding sites

SRY GTTGTTT

20 GATA CATCAGC

OCT-X GCTGCTTTGCATAG

(residues 124-137 of SEQ ID NO: 9)

NF-KB GAGGCTGCCCBU

(residues 240-249 of SEQ ID NO: 9)

25 Alignment of B29 DHS4.4 3' enhancer PCR fragment (297 bp (SEQ ID NO: 9)) in pGEM-4.4enh #8 with sequence of the human CD79B/GH-1 Intergenic region (Accession AB062674 (SEQ ID NO: 10)). The consensus sequence is disclosed as SEQ ID NO: 9.

1	10	20	30	40	50	60	70	80	90	100	110	120	130
GGGAACAGCTGCCAGCTGGGAGACCAAGTGCACCTGCACGTGCAAGCTCCCTCCAAAGCCAGGCTGTCTCCACTTCTGTGACCCCTGGAGGGAATCCITTCGAGGCCCTCTGTCTAATTCCTG													
Consensus													
131	140	150	160	170	180	190	200	210	220	230	240	250	260
CTCTGAATTCAGCAAGGAGGACCTATGCTGTGGGAGCTGCCAGTTTAACTGGGGAATCAAGACCAGCACAGGGAACTAGTGAGAACAGTGCCTAATTTTCCAGATTCCTCTGGAAATTCACAGGTGG													
Consensus													
261	270	280	290	300	310	320	330	340	350	360	370	380	390
GGCAGGTGGGTAAGCCCCACGCTGCAGTTTCAGTAAATCTCTCCACCACTGGGCAAGGCTGGCCCAAGCCAGGCGGCCCTGTGTGTTTTCCCAAGTCTCTGGACTGTGGAGGAAACCAGGTTGT													
Consensus													
391	400	410	420	430	440	450	460	470	480	490	500	510	520
TTTGACATCAGCCTCTACTGAGCCGGAGCCCTTCCCTTCTGCTGCTTTTCACTAGTAGCACTGATTCATCTCTACCTCCACAGGGACCTAGGACCCGGGTAGATGTGAGAGGAGGCTTCACTTC													
Consensus													
521	530	540	550	560	570	580	590	600	610	620	630	640	650
TCCCCAAGCAGGATCTCCACCTGTGAGGCTGCCCTGGGAGGCTGGCTTACCTAAAGACTTTTAAACACTTGAACAACACAGTTTCCCTGAGACTTTTGAAGCTCTTGTGTTTTAATTTA													
Consensus													
651	660	670	680	690	700	710	720	730	740	750	760	770	780
TTTTAATTTAATTTAATTTAATTTAATTTAATTTAATTTGACAGAGTCTCACTCTGTGTGCCAGACTGGAGTGCAGTGGCACCAATCTCCGCTCACTGCAACCTCCGCTCTCCTGAGTTCAGCAATTCCTCCT													
Consensus													
781	790	800											
GCCTCAGCCTCCAAAGTACC													
Consensus													

Step 2: Subcloning of minimal B29 promoter into pCpG-LacZ (Invivogen)—creates B29-5 #3.

The minimal -167 human B29 promoter was amplified from a commercial plasmid bearing the full length human B29 promoter (pDrive-hB29; Invivogen) using the following primers: forward 5'-CCA ACTAGTGCGACCGCCAAACCTTAGC-3' (SEQ ID NO: 11); reverse: 5'-CAA AAGCTTGACAACGTCCGAGGCTCCTTGG-3' (SEQ ID NO: 12). The resulting PCR fragment was digested with SpeI and HindIII and ligated into the SpeI and HindIII sites of the pCpG-LacZ vector (Invivogen) to create B29-5 #3. Sequence of B29 minimal promoter PCR fragment (188 bp) in B29-5 #3 (SEQ ID NO: 13)

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GCGACCGCCAAACCTTAGCGGCCAGCTGACAAAAGCCTGCCCTCCCCCA

GGGTCCCCGGAGAGCTGGTGCCTCCCCTGGGTCCCAATTTGCATGGCAGG

AAGGGCCTGGTGAGGAAGAGCGGGGAGGGGACAGGCTGCAGCCGGTG 20

CAGTTACACGTTTTCTCCAAGGAGCCTCGGACGTTGTC

Alignment of B29 minimal promoter PCR fragment (B29min) in B29-5 #3 (SEQ ID NO: 13) with full length human B29 promoter from pDrive-hB29 (SEQ ID NO: 14). The consensus sequence is disclosed as SEQ ID NO: 13.

25

1	10	20	30	40	50	60	70	80	90	100	110	120	130
B29_prom	CCTGCAGGCCCACTAGTAAACGGAGGGTGTGAGGAGAGTGAGAGGCGCACCGACGATTTAGCATCTCTTCTCTCTGCGGGTTCGAGGATGAGAGACAAAAAAGAAGCTGCCAGGAAC												
B29_min	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
Consensus	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
131	140	150	160	170	180	190	200	210	220	230	240	250	260
B29_prom	ATAAAATTCAGAGGGCTCAGCTGCAGGGCTGAGGTCTGCAAGCATGCTGTGTACACTTGTGCATGTTGCTCCCTGCACAAGGGCATCTCTGAAGGGGTGCACCTGGACCCAGGGCAGGGCCGCAAGGT												
B29_min	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
Consensus	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
261	270	280	290	300	310	320	330	340	350	360	370	380	390
B29_prom	GAGTTTATATCAGTTCCCTGAGCACTGTGGCTCCATCCAGCACTCTGAGGACAGGCAGGATACAGCTGGAGGACCTGAGGGTCCCTCCACACCACTCTCCCTGCTCCCTGCCCCAAGACCCCTGGACCTGCAG												
B29_min	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
Consensus	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
391	400	410	420	430	440	450	460	470	480	490	500	510	520
B29_prom	ACAACAATTCACCGCACTCAGAGTCCCACAGTTAAGAACTCCCTGAAGAAGCCCCAGTGGCTGGTGGTGGATTTTCCGAAAAGCTGTCTCCACCCTACATCCACCCTGTTTGGCAGCCCTACATACTCT												
B29_min	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
Consensus	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
521	530	540	550	560	570	580	590	600	610	620	630	640	650
B29_prom	TTCACAGATGAGGAAGGGAGCCCTCTACCAAGACTGGACTGAATCTTCTCCAGTGGCTGCCACACTGACCTGCTTTGGTCCAGAACCTCTCTGGTCCCATCTCCACAGGGTTCAGGACAGAGG												
B29_min	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
Consensus	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
651	660	670	680	690	700	710	720	730	740	750	760	770	780
B29_prom	ATGGTGCCTGCACCTCAGCCAAAGAGGCTCTGCTCTGGGCCCTCCAGATGCCCTGACCTGGGTCTGTGGTGCCTGTCTCTTTCAGTGTCTCCCTTCCCGCTGGGTGAGGAATAGTTTCAGGACAGAGG												
B29_min	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
Consensus	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
781	790	800	810	820	830	840	850	860	870	880	890	900	910
B29_prom	AGCTAAGTTCAGGTTCAITTCATAGACAGGTGCCCTATTTCGCTCACGGCCCAAGAAATAGAGACTTTGCCGGCTCGGCCCTTCGGGAGTTGGCAGACGGCAGAGGGAGGCTGGCTGGCCAGGGGATGA												
B29_min	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
Consensus	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
911	920	930	940	950	960	970	980	990	1000	1010	1020	1030	1040
B29_prom	CCACCGTGGGTTAAGCACAGACAGAGGGGAGCACAGGCTTCCCCAGAAAGACTGAGAGGCCCCCCAGAGGATCCACAGAGGACCCCAAGCTGTGTGCTGCCCAAAACCTTAGCGGC												
B29_min	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
Consensus	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
1041	1050	1060	1070	1080	1090	1100	1110	1120	1130	1140	1150	1160	1170
B29_prom	CCAGCTGACAAAACCTGCTCCCTCCAGGGTCCCCGGAGAACTGGTGCCTCCCTGGTCCCAATTTGCATGGCAGAAAGGAGCCCTGGTGAAGGAGGGGGAGGGGACAGGTACAACCCGGTGCAG												
B29_min	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
Consensus	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
1171	1180	1190	1200	1210	1220	1230	1237						
B29_prom	TTACACGTTTCTCCTCAAGGAGCTCGAACGTTTGTACAGGGTTTGGGTCGGGGACAGAGCGGTGAC												
B29_min	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												
Consensus	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----												

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Step 3: Subcloning of HA-eIF5A1<sup>K50R</sup> into B29-5#3 vector—creates pB29-eIF5A1K50R\_7.

HA-eIF5A1<sup>K50R</sup> was amplified by PCR using the pHM6-eIF5A1<sup>K50R</sup> as a DNA template and the following primers: forward 5'-CGCCATGGACATGTACCCTTACGACGTC-CCAGACTACGCTGCAGATGATTTGGACTT CGAG-3' (SEQ ID NO: 15) and reverse 5'-CGC GCTAGCCAGTTATTTTGGCCATCGCC-3' (SEQ ID NO: 16). The resulting PCR fragment was digested with NcoI and NheI and subcloned into the NcoI and NheI sites of B29-5 #3 to replace LacZ.

Sequence of HA-eIF5A1<sup>K50R</sup> PCR fragment (497 bp) in pB29-eIF5A1K50R\_7 (SEQ ID NO: 17).

ACATGTACCCTTACGACGCTCCAGACTACGCTGCAGATGATTTGGACTTC  
 GAGACAGGAGATGCAGGGCCCTCAGCCACCTTCCAATGCAGTGCTCAGC  
 ATTACGTAAGAATGGTTTTTGGTGTCTCAAGGGCCGGCCATGTAAGATCG  
 TCGAGATGTCTACTTCGAAGACTGGCAGGCATGGCCATGCCAAGGTCCAT  
 CTGGTTGGCATTGATATTTTACTGGGAAGAAATATGAAGATATCTGCC  
 GTCGACTCATAACATGGATGTCCCAACATCAAAGGAATGATTTCCAGC  
 TGATTGGCATCCAGGATGGGTACCTATCCCTGCTCCAGGACAGTGGGGAG  
 GTACGAGAGGACCTTCGTCTGCCTGAGGGAGACCTTGGCAAGGAGATTGA  
 GCAGAAGTATGACTGTGGAGAAGAGATCCTGATCACAGTGTCTGCCCA  
 TGACAGAGGAGGAGCTGTTGCAATCAAGGCGATGGCAAATAACTG

Translation of HA-eIF5A1<sup>K50R</sup> PCR fragment in pB29-eIF5A1K50R\_7 (SEQ ID NO: 18) HA epitope  
 eIF5A1<sup>K50R</sup>  
 K50R

MDMYPYDVPDYAADLDFETGDAGASATFPNQCSALRKNQGFVVLKGRPCKI  
VEMSTSKTGKHHGHAKVHLVGLIDIFTGKKYEDICPSTHNMVDPNIKRNDF  
QLIGIQDGYLSLLQDSGEVREDLRLPEGDLGKEIEQKYDCGEEILITVLSA  
MTEEA AVAIKAMAK

Alignment of HA-eIF5A1<sup>K50R</sup> PCR fragment in pB29-eIF5A1K50R\_7 (SEQ ID NO: 18) with human eIF5A1 (Accession #NP\_001961 (SEQ ID NO: 19)). The consensus sequence is disclosed as residues 12-165 of SEQ ID NO: 18.

	1	10	20	30	40	50	60	70	80	90	
eIF5A	MADDLDFETGDAGASATFPNQCSALRKNQGFVVLKGRPCKI VEMSTSKTGKHHGHAKVHLVGLIDIFTGKKYEDICPSTHNM										
HA-5A_K50R	MDMYPYDVPDYAADLDFETGDAGASATFPNQCSALRKNQGFVVLKGRPCKI VEMSTSKTGKHHGHAKVHLVGLIDIFTGKKYEDICPSTHNM										
Consensus	..... aADDLDFETGDAGASATFPNQCSALRKNQGFVVLKGRPCKI VEMSTSKTGKHHGHAKVHLVGLIDIFTGKKYEDICPSTHNM										
	100	110	120	130	131	140	150	160	165		
eIF5A	DVPNIKRNDFQLIGIQDGYLSLLQDSGEVREDLRLPEGDLGKEIEQKYDCGEEILITVLSAMTEEA AVAIKAMAK										
HA-5A_K50R	DVPNIKRNDFQLIGIQDGYLSLLQDSGEVREDLRLPEGDLGKEIEQKYDCGEEILITVLSAMTEEA AVAIKAMAK										
Consensus	DVPNIKRNDFQLIGIQDGYLSLLQDSGEVREDLRLPEGDLGKEIEQKYDCGEEILITVLSAMTEEA AVAIKAMAK										

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vector was then ligated to a synthetic linker with EcoRI sticky ends to create a promoterless vector with a new multiple cloning site (pCpG-Linker4). SEQ ID NOS 20-21 are disclosed below, respectively, in order of appearance.



Synthetic Linker, Linker4 with two Eco RI sticky ends  
 Sequence of region surrounding new multiple cloning site in pCpG-Linker4 (SEQ ID NO: 22)

GGCATGTGAACTGGCTGTCTTGGTTTTTCATCTGTACTTCATCTGCTACCT  
 CTGTGACCTGAAACATATTTATAATTCCATTAAGCTGTGCATATGATAGA  
 20 TTTATCATATGTATTTTCTTAAAGGATTTTTGTAAGAACTAATTGAATT  
 GATACCTGTAAAGTCTTTATCACACTACCCAATAAATAATAAATCTCTTT  
 25 GTTCAGCTCTCTGTTTCTATAAATATGTACAAGTTTTATTGTTTTTAGTG  
 GTAGTGATTTTATCTCTTTCTATATATATACACACACATGTGTGCATTC  
 ATAAATATATACAATTTTATGAATAAAAAATATTAGCAATCAATATTG  
 AAAACCACTGATTTTTGTTTTATGTGAGCAAACAGCAGATTAAAGGAATT  
 30 CTCGAGTCATCGATAAGCGGCCGCAGACGCGTAATTCAGTCAATATGTTC  
 ACCCCAAAAAGCTGTTTGTAACTTGCCAACCTCATTCTAAAATGTATA  
 TAGAAGCCCAAAAGACAATAACAAAAATATTCTTGTAGAACAAAAATGGGA  
 35 AAGAATGTCCACTAAATATCAAGATTTAGAGCAAAGCATGAGATGTGTG  
 GGGATAGACAGTGAGGCTGATAAAATAGAGTAGAGCTCAGAAACAGACCC  
 ATTGATATATGTAAGTGACCTATGAAAAAATATGGCATTTTACAATGGG  
 AAAATGATGATCTTTTTCTTTTTTAGAAAAACAGGGAAATATATTTATAT  
 40 GTAAAAATAAAAGGGAACCCATATGTCATACCATAACACAAAAAAAAT

Step 4: Creation of new multiple cloning site in pCpG-mcs (Invivogen)—creates pCpG-Linker4.

The pCpG cloning vector, pCpG-mcs G2 (Invivogen), was digested with EcoRI to remove the mammalian expression cassette containing the mCMV enhancer, the hEF1 promoter, the synthetic intron, the multiple cloning site, and the SV40 polyadenylation signal. The EcoRI-digested pCpG-mcs G2

60 - continued  
 ATCCAGTGAATTATAAGTCTAAATGGAGAAGGCAAACTTTAATCTTTTA  
 GAAAATAATATAGAAGCATGCCATCAAGACTTCAGTGTAGAGAAAAATT  
 65 CTTATGACTCAAAGTCCTAACCACAAAGAAAAGATTGTTAATTAGATTGC



-continued

ATGAATATTAAGACTTATTTTTTAAAATTA AAAAACCATTAAGAAAAGTCA  
GGCCATAGAATGACAGAAAATATTTGCAAC

βGlo MAR (nucleotides 1-380); EcoRI recognition sequence (nucleotides 396-401); XhoI recognition sequence (nucleotides 401-406); ClaI recognition sequence (nucleotides 409-414); NotI recognition sequence (nucleotides 417-424); MluI recognition sequence (nucleotides 427-432); IFNβ S/MAR (nucleotides 438-1,030).

Step 5: Subcloning of B29 DS4.4 3' enhancer into pCpG-Linker4—creates pCpG-DHS4.4.

The B29 DHS4.4 3' enhancer was amplified by PCR using pGEM-4.4enh #8 as a template and the following primers: forward 5'-GAA GCGGCCGCACCACCCTGGGCCAGGCTGG-3' (SEQ ID NO: 23); and reverse 5'-CC ACGCGTAGAGGTGTTAAAAAGTCTTTAGGTAAAG-3' (SEQ ID NO: 24). The resulting PCR fragment was digested with NotI and MluI and ligated into the NotI and MluI sites in the new multiple cloning site of pCpG-Linker4 to create pCpG-DHS4.4.

>pCpG-DHS4.4 full-length sequences (2,282 bp (SEQ ID NO: 25))

TTAATTA AAAATTATCTCTAAGGCATGTGAACTGGCTGTCTTGGTTTTTCAT  
CTGTACTTCATCTGCTACCTCTGTGACCTGAAACATATTTATAATTCAT  
TAAGCTGTGCATATGATAGATTTATCATATGTATTTTCCTTAAAGGATTT  
TTGTAAGAACTAATTGAATTGATACCTGTAAAGTCTTTATCACACTACCC  
AATAAATAATAAATCTCTTTGTTTCAGCTCTCTGTTTCTATAAATATGTAC  
AAGTTTTATTGTTTTTAGTGGTAGTGATTTTATTCTCTTTCTATATATAT  
ACACACACATGTGTGCATTATAAATATATAAATTTTATGAATAAAAA  
ATTATTAGCAATCAATATTGAAAACCACTGATTTTGTTTATGTGAGCAA  
ACAGCAGATTAAGGAATTCTCGAGTCATCGATAAGCGGCCGACCACC  
CTGGGCCAGGCTGGGCCAAGCCAGGCGGCCCTGTGTTTTCCCGAGTCTC  
TGGGCTGCTGGAGGGAACCAGGTTGTTTTGGCATCAGCCTCTACTGAGCC  
GGAGCCCTTCCTTTCTGCTGCTTTGCATAGTGGCACTAATTCGTCCTC  
CTACCTCCACCAGGGACCTAGGCAGCCGGGTAGATGGTGGGAGGAGGCTT  
CACTTCTCCCCAAGCAGGGTCTCCACCTGCTTGAGGCTGCCCTGGGTTG  
GGGAGGCCTTGGCTTTACCTAAAGACTTTTAAACACCTCTACGCCTAAT  
TCAGTCAATATGTTTACCCAAAAAAGCTGTTTGTAACTTGCCAACCTC  
ATTCTAAAATGTATATAGAAGCCAAAAGACAATAACAAAAATATTCTTG  
TAGAACAAAATGGGAAAGAATGTTCCACTAAATATCAAGATTTAGAGCAA  
AGCATGAGATGTGTGGGGATAGACAGTGAGGCTGATAAATAGAGTAGAG  
CTCAGAAACAGACCCATTGATATATGTAAGTGACCTATGAAAAAATATG  
GCATTTTACAATGGGAAAATGATGATCTTTTTCTTTTTTAGAAAAACAGG  
GAAATATATTTATATGTAAAAATAAAAGGGAACCCATATGTCATACCAT  
ACACACAAAAAATTCAGTGAATTATAAGTCTAAATGGAGAAGGCAAAA  
CTTTAAATCTTTTAGAAAAATAATATAGAAGCATGCCATCAAGACTTCAGT

-continued

GTAGAGAAAAATTTCTTATGACTCAAAGTCCTAACCCACAAAGAAAAGATT  
GTTAATTAGATTGCATGAATATTAAGACTTATTTTTTAAAATTA AAAAAC  
5 ATTAAGAAAAGTCAGGCCATAGAATGACAGAAAATATTTGCAACACCCCA  
GTAAAGAGAATTGTAATATGCAGATTATAAAAAGAAGTCTTACAAATCAG  
TAAAAAATAAACTAGACAAAAATTTGAACAGATGAAAGAGAACTCTAA  
10 ATAATCATTACACATGAGAACTCAATCTCAGAAATCAGAGAATCATCAT  
TGCATATACACTAAATTAGAGAAAATATTAAGGCTAAGTAACATCTGTG  
GCTTAATTA AAAACAGGTAGTTGACAATTA AACATTGGCATAGTATATCTG  
CATAGTATAATACAACCTCACTATAGGAGGGCCATCATGGCCAAGTTGACC  
15 AGTGCTGTCCAGTGCTCACAGCCAGGGATGTGGCTGGAGCTGTTGAGTT  
CTGGACTGACAGGTTGGGGTCTCCAGAGATTTTGTGGAGGATGACTTTG  
CAGGTGTGGT CAGAGATGATGTCACCCTGTTTCATCTCAGCAGTCCAGGAC  
20 CAGGTGGTGCCTGACAACACCCTGGCTTGGGTGTGGGTGAGAGGACTGGA  
TGAGCTGTATGCTGAGTGGAGTGAGGTGGTCTCCACCAACTTCAGGGATG  
CCAGTGGCCCTGCCATGACAGAGATTGGAGAGCAGCCCTGGGGGAGAGAG  
25 TTTGCCCTGAGAGACCCAGCAGGCAACTGTGTGCACTTTGTGGCAGAGGA  
GCAGGACTGAGGATAACCTAGGAAACCTTAAAACCTTTAAAAGCCTTATA  
TATTCTTTTTTTCTTATAAACTTAAAACCTTAGAGGCTATTTAAGTTG  
30 CTGATTTATATTAATTTTATTGTTCAAACATGAGAGCTTAGTACATGAAA  
CATGAGAGCTTAGTACATTAGCCATGAGAGCTTAGTACATTAGCCATGAG  
GGTTTAGTTCATTAAACATGAGAGCTTAGTACATTAAACATGAGAGCTTA  
35 GTACATACTATCAACAGGTTGAACTGCTGATC

βGlo MAR (nucleotides 1-400); EcoRI recognition sequence (nucleotides 416-421); XhoI recognition sequence (nucleotides 421-426); ClaI recognition sequence (nucleotides 429-434); NotI recognition sequence (nucleotides 437-444); DHS4.4 (nucleotides 445-741); MluI recognition sequence (nucleotides 742-747); IFNβ S/MAR (nucleotides 753-1,569).

Step 6: Subcloning of B29 promoter+HA-eIF5A1<sup>K50R</sup>+SV40 pA expression cassette into pCpG-DHS4.4—creates pExp-5.

The B29-eIF5A1 expression cassette containing the minimal B29 promoter, the synthetic intron, HA-eIF5A1<sup>K50R</sup>, and the SV40 pA, was amplified from pB29-eIF5A1K50R\_7 (Step 3) by PCR using the following primers: forward 5'-GT-TATCGATACTAGTGCAGCCGCAAACC-3' (SEQ ID NO: 26); and reverse 5'-CAAGCGGCCGCCATACCA-CATTTGTAGAGGTTTTAC-3' (SEQ ID NO: 27). The resulting PCR fragment was digested with ClaI and NotI and subcloned into the ClaI and NotI sites in the multiple cloning site of pCpG-DHS4.4 to create pExp-5.

Step 7: Replacement of HA-eIF5A1<sup>K50R</sup> in pExp-5 with non-HA eIF5A1<sup>K50R</sup> creates final vector, pExp5A.

The pExp-5 plasmid was digested with NcoI and NheI to remove HA-eIF5A1<sup>K50R</sup>. A non-HA-tagged eIF5A1<sup>K50R</sup> PCR fragment was amplified from pHM6-eIF5A1<sup>K50R</sup> by PCR using the following primers: forward 5'-CACCATG-GCAGATGATTTGGACTTC-3' (SEQ ID NO: 28); and reverse 5'-CGCGCTAGCCAGTTATTTTGCCATCGCC-3' (SEQ ID NO: 16). The resulting PCR product was digested with NcoI and NheI and ligated into the NcoI and NheI sites

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of B29-5 #3 to generate B29-K50R. B29-K50R was digested with NcoI and NheI and the 470 bp eIF5A1<sup>K50R</sup> fragment was gel purified and ligated to NcoI/NheI-digested pExp-5 to generate the final expression vector, pExp5A.

## Example 18

## Testing of pExp5A

Various cell lines were transfected with plasmids using Lipofectamine 2000 and expression of HA-eIF5A1K50R was determined 24 hours following transfection by Western blotting with an anti-HA antibody (Roche). Different cell lines tested were P3X63Ag8.653 (mouse B lymphoblast—myeloma), KAS (human myeloma), HepG2 (human liver hepatocellular carcinoma), T24 (human bladder carcinoma); HT-29 (human colorectal adenocarcinoma), HEK-293 (human embryonic kidney cells), PC3 (human prostate adenocarcinoma); HeLa (human cervical adenocarcinoma), and A549 (lung carcinoma). See FIG. 24.

pExp-5 plasmid expresses HA-eIF5A1<sup>K50R</sup> in both human and mouse myeloma cell lines at comparable levels to a plasmid with the constitutive EF1 promoter (CpG-eIF5A1<sup>K50R</sup>). However expression of HA-eIF5A1<sup>K50R</sup> driven by pExp-5 is limited in non-B cell lines compared to expression by a constitutive promoter. The one exception was in HEK-293 cells, a human embryonic kidney cell line where high levels of HA-eIF5A1<sup>K50R</sup> expression was observed following pExp-5 transfection—this may be due to the embryonic nature of the cell line; at this time we do not know if pExp-5 expresses in adult kidney cells. The final plasmid for use in toxicity studies and clinical trial will be a version of pExp-5 in which HA-eIF5A1<sup>K50R</sup> has been replaced by non-HA tagged eIF5A1<sup>K50R</sup> (pExp5A). pExp-5 contains HA-tagged eIF5A1<sup>K50R</sup> under the control of the minimal human B29 promoter/enhancer; expression of HA-eIF5A1<sup>K50R</sup> was compared to that driven by plasmids with constitutive expression as well as to a plasmid containing the full-length B29 promoter

## Example 19

## Formation of In vivo JETPEI™ Nanoparticle

This example given is for formation of the in vivo JETPEI™ nanoparticle complex for injection into 20 g mouse for a dose of 1.5 mg/kg (0.1 mL)—1.5 mg/kg=1.0 mg pExp5A/kg+0.5 mg h5A1/kg—DNA: siRNA ratio=2:1.

Dilute plasmid DNA and siRNA into a total volume of 25 ml. Use sterile water to adjust the volume. \*Dilute 20 mg of plasmid DNA (10 ml at 2 mg/ml) and 10 mg of siRNA (10 ml at 1 mg/ml) into a total volume of 25 ml. Use sterile water to make up difference. Adjust the volume of DNA solution to 50 ml 5% glucose by adding 25 ml of 10% glucose (provided with PEI kit). Vortex gently and centrifuge briefly. Dilute in vivo JETPEI™ into a total volume of 25 ml of water. \*Dilute 3.6 ml of in vivo JETPEI™ into a total volume of 25 ml of water. Adjust final volume to 50 ml with 10% glucose to end up with a final concentration of 5% glucose. Vortex gently and

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centrifuge briefly. Immediately add 50 ml of diluted PEI to the 50 ml of diluted DNA (do not reverse the order!). Vortex briefly and immediately spin down.

After formation the complex is stable for 8 to 10 hours. The N/P ratio of the complex should be 6. The N/P ratio is the ratio of the number of positively charged nitrogen residues of in vivo-jetPEI to the number of negatively charged phosphate residues of DNA and siRNA. DNA and siRNA contain the same number of phosphate groups per gram. The N/P ratio is therefore a measure of the ionic balance within the complex. Increasing the N/P ratio of the complex can increase the toxicity of the complex. In vivo JET-PEI is provided as a 150 mM solution (expressed as nitrogen residues) while DNA contains 3 nmoles of anionic phosphate in 1 mg. The final concentration of DNA in the final volume should not exceed 0.5 mg/ml. The DNA should be of high quality and prepared in water. In vivo-jetPEI and 10% glucose should be brought to room temperature prior to use.

## Example 20

## Dose Range-Finding and Repeat Dose Studies with Intra-Venous SNS01 and SNS-EF1/UU in Mice

SNS01 is one embodiment of the present invention—it is a cancer therapy biologic targeted to the treatment of multiple myeloma. SNS01 is comprised of three components: a DNA vector expressing a pro-apoptotic mutant of eIF5A (see FIG. 22); an siRNA that targets the native eIF5A that promotes growth/anti-apoptosis of cancer cells (see the sequence in FIG. 25); and a synthetic polymer called polyethylenimine (In vivo-jetPEI; Polyplus Transfection Inc.) that acts as a delivery vehicle.

The purpose of the studies was to determine the maximum tolerated dose and the feasibility of long-term administration of therapeutic doses of intra-venous SNS01 into mice. Two separate studies were performed. The maximum tolerated dose (Study ID MTD) study was an 8-day study in which mice received two intra-venous doses of increasing amounts of SNS01 (from 2.2 mg/kg to 3.7 mg/kg) and toxicity was assessed by monitoring clinical signs, body weight, organ weight and liver enzymes. The 9-week repeated dose study (Study ID:EX6) was a study designed to assess toxicity following long-term administration of twice-weekly therapeutic doses (1.5 mg/kg) of SNS-EF1/UU and as well as its various components. SNS-EF1/UU is a preclinical version of SNS01 and differs mainly in that expression of eIF5A<sup>K50R</sup> is driven by a constitutive promoter (one that expresses in all tissues at all times) rather than a B-cell-specific promoter as in the SNS01 complex. The use of the B cell-specific B29 promoter in SNS01 was designed to enhance the safety of the therapeutic by limiting expression of the pro-apoptotic eIF5A mutant to cells of B-cell origin, including myeloma cells. The EX6 study also included a group of mice that were dosed with a mouse-specific eIF5A siRNA to determine whether there were any toxic effects of suppressing eIF5A in mouse tissues. Toxicity in the repeated dose study was assessed by monitoring clinical signs, body weight, hematology, liver enzymes, as well as histopathology.

Study ID	Animal Model	Injection Schedule	Duration of Treatment	Test Article
MTD	CD-1 (female)	Twice weekly Intra-venous	8-Days (2 injections)	SNS01

-continued

Test Article	Plasmid DNA	siRNA	Vehicle	Material Grade
SNS01 <sup>a</sup>	pExp5A <sup>c</sup> (eIF5A <sup>K50R</sup> expression driven by B29 B-cell- specific promoter)	eIF5A siRNA (dTdT overhang <sup>d</sup> ) human-specific eIF5A siRNA	In vivo-jetPEI™	GLP-grade materials
SNS-EF1/UU <sup>b</sup>	pCpG-HA- eIF5A <sup>K50R</sup> (HA-eIF5A <sup>K50R</sup> expression driven by ubiquitous EF1 promoter)	h5A1 (UU overhang <sup>d</sup> ) human-specific eIF5A siRNA	In vivo-jetPEI™	Research-grade materials

<sup>a</sup>SNS01 contains GLP-grade materials and is being developed for the clinic

<sup>b</sup>SNS-EF1/UU is a test article used in preclinical research that led to the development of SNS01

<sup>c</sup>the pExp5A plasmid is RNAi-resistant due to the fact that the plasmid contains only the open reading frame of eIF5A while the eIF5A siRNA (h5A1) targets the 3' UTR of eIF5A

<sup>d</sup>the sequence of the SNS01 eIF5A siRNA and the h5A1 siRNA are identical except for the presence of a dTdT overhang rather than a UU overhang on the 3' terminal ends of the siRNA; the dTdT overhang does not affect the target selectivity or efficacy of the siRNA but has been proposed to enhance stability

Preclinical experiments have indicated that SNS01 is therapeutic at doses of 0.75 mg/kg to 1.5 mg/kg (Study EX9). In the 8-day dose range-finding study (Study ID: MTD) significantly higher doses than the therapeutic range was tested to determine the upper limit of the dose range. Twice-weekly intra-venous doses of the test article was well tolerated at the

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was also included in this study. No toxic effects related to chronic administration of the mouse eIF5A siRNA were observed over the 9-week period. These results indicate that therapeutic doses of SNS01 and SNS-EF1/UU are non-toxic to mice even when administered over long periods.

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Test Article and Vehicle

Test Article	Manufacturer of Plasmid DNA component	Manufacturer of siRNA component	Manufacturer of PEI component	Formation of Test Article
SNS01	VGXI Lot # pExp5A.08L007	Avecia	Polyplus Transfection Inc GLP-Grade	Components were combined with 10% glucose to form nano-complexes; complexes were injected within 2-4 hours
SNS-EF1/UU	Qiagen EndoFree Plasmid Mega Kit <0.1 EU/μg DNA	ThermoScientific Dharmacon RNAi Technologies	Polyplus Transfection Inc. Research-Grade	Components were combined with 10% glucose to form nano-complexes; complexes were injected within 2-4 hours
Storage Conditions (Stability)	-20° C. (≥1 year)	-20° (≥1 year)	4° (≥1 year)	Room Temperature (≥6 hours)

lower dose levels of 2.2 mg/kg and 2.9 mg/kg although one mouse reached morbidity at the 2.9 mg/kg and was euthanized. Doses at 3.3 mg/kg or above resulted in survival rates of approximately 20-25%. Therefore, the maximum tolerated dose is between 2.2 mg/kg and 2.9 mg/kg and is well above the therapeutic range of 0.75 mg/kg to 1.5 mg/kg.

In the 9-week repeated dose study (Study ID: EX6) the mice received twice weekly tail vein injections of therapeutic doses (1.5 mg/kg) of SNS-EF1/UU and no test article-related toxic effects were observed over the period of the study. The DNA and siRNA were also tested separately in this study and both were well tolerated by the mice. Since the human eIF5A siRNA is not active in mice, a mouse eIF5A-specific siRNA

55 Test Systems and Study Designs

All aspects of this study were conducted in accordance with the guidelines set out by the University of Waterloo Animal Care Committee (Waterloo, Ontario, Canada) as established by the Canadian Council on Animal Care and the Province of Ontario Animals for Research Act.

The CD-1 and BALB/c mice were obtained from Charles River Laboratories (Quebec, Canada). Mice from both studies received the test article twice-weekly via tail vein intra-venous injections. Slow injections (~2-3 minutes) were used to deliver volumes greater than 0.2 ml.

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The mice for the 8-day study were approximately 6-9 weeks old at the start of study. The mice for the 9-week repeated dose study were approximately 5-6 weeks old at the initiation of the study.

STUDY ID: MTD						
Group	No. Animals (female)	Test Article	N/P ratio <sup>a</sup>	Dose level (mg/kg)	Injection Volume	Total # injections
MTD-C	5	5% Glucose	—	—	0.33 mL	2
MTD-PA	5	SNS01	6	2.2 mg/kg	0.20 mL	2
MTD-PB	5	SNS01	6	2.9 mg/kg	0.27 mL	2
MTD-PC	4	SNS01	6	3.3 mg/kg	0.30 mL	1 <sup>b</sup>
MTD-PD	5	SNS01	6	3.7 mg/kg	0.33 mL	1 <sup>b</sup>

<sup>a</sup>N/P ratio = ratio of positively-charged nitrogens on PEI to the negatively-charged phosphate groups of the nucleic acids  
<sup>b</sup>due to toxicity the surviving mice were not given a second injection

STUDY ID: EX6						
Group	No. Animals (female)	Test Article	N/P ratio	Dose level (mg/kg)	Injection Volume	Total # injections
Ex6-G1	4	5% Glucose	—	—	0.10 mL	20
Ex6-G2	5	Vehicle Control <sup>a</sup>	8	1.5 mg/kg	0.10 mL	20
Ex6-G3	5	siRNA <sup>b</sup>	8	1.5 mg/kg	0.10 mL	20
Ex6-G4	6	DNA <sup>c</sup>	8	1.5 mg/kg	0.10 mL	20
Ex6-G5	4	SNS-EF1/UU	8	1.5 mg/kg	0.10 mL	20
Ex6-G6	6	Mouse siRNA <sup>d</sup>	8	1.5 mg/kg	0.10 mL	20

<sup>a</sup>PEI complex containing a non-expressing plasmid (same vector background as pExp5A) and a non-targeting siRNA

<sup>b</sup>PEI complex containing a non-expressing plasmid (same vector background as pExp5A) and the h5A1 siRNA

<sup>c</sup>PEI complex containing pCpG-HA-eIF5A<sup>K50R</sup> plasmid and a non-targeting siRNA

<sup>d</sup>PEI complex containing a non-expressing plasmid (same vector background as pExp5A) and a mouse-specific eIF5A siRNA (the human eIF5A siRNA is not active in mouse)

#### 8-Day Maximum Tolerated Dose Study (MTD)

The two-dose 8-day study was a dose range-finding study designed to determine the maximum tolerated dose of SNS01. The dose range was 2.2 mg/kg to 3.7 mg/kg and is well above the therapeutic dose range of 0.75 mg/kg to 1.5 mg/kg. At the lowest dose (2.2 mg/kg) of SNS01 no clinical signs of toxicity were observed except for one mouse that exhibited slightly ruffled fur and decreased activity that resolved within one hour. No clinical signs of toxicity were observed following the 2<sup>nd</sup> injection of 2.2 mg/kg of SNS01. All the mice maintained their weight throughout the study. No macroscopic changes in the organs were observed. The organ weight to body weight ratios were unchanged from the control group except for a modest increase in the ratio of the liver weight:body weight ratio. However, since an increase in this ratio was not observed in any of the higher dose level groups it is unlikely to be related to the test article.

Four out of five mice tolerated 2.9 mg/kg of SNS01 with no clinical signs of toxicity. However, one mouse experienced convulsions and mild respiratory distress within 1 hour of injection and had to be humanely euthanized. No clinical signs of toxicity were observed following the 2<sup>nd</sup> injection of SNS01 in the remaining mice. The mice maintained their weight throughout the study and no macroscopic changes in the organs or changes in the organ weight to body weight

ratios were observed. There was a slight increase in the serum levels of ALT following two doses of 2.9 mg/kg SNS01.

As expected, doses of SNS01 at or above 3.3 mg/kg were not well tolerated and in both groups all mice but one had to be humanely euthanized. In all cases where mice were humanely euthanized due to morbidity the clinical signs appeared within 1 hour of injection and were consistent with other reported studies using high doses of PEI. The surviving mice of the 3.3 mg/kg and 3.7 mg/kg recovered completely within 4 hours after the injection and maintained their weight throughout the study, although they did not receive a 2<sup>nd</sup> dose. The maximum tolerated dose of SNS01 therefore appears to be between 2.2 mg/kg and 2.9 mg/kg.

#### 9-Week Repeated Dose Study (EX6)

The purpose of the 9-week repeated dose study was to assess the safety of chronic administration of therapeutic doses (1.5 mg/kg) of SNS-EF1/UU, a complex that was used for preclinical studies during development of SNS01. SNS-EF1/UU does not differ significantly from SNS01, the major difference being that the materials are research-grade and that eIF5A<sup>K50R</sup> expression is driven by the constitutive human EF1 promoter that is active in all cell types. Although SNS01 uses a B-cell-specific promoter to drive eIF5A<sup>K50R</sup> expression, the use of a constitutive promoter in this safety study allows for the assessment of toxicity resulting from the accumulation of the mutant eIF5A<sup>K50R</sup> protein in non-B-cell tissues. Another aspect of the 9-week repeated dose study was the inclusion of groups to test the safety of the individual components of SNS-EF1/UU. The DNA group (Ex6-G3) was dosed with a complex containing the eIF5A plasmid and a non-targeting control siRNA while the siRNA group (Ex6-G4) was dosed with a complex containing the human eIF5A (h5A1) siRNA and a non-expressing plasmid. Since the test article SNS-EF1/UU contains a human eIF5A siRNA that will not affect expression of the endogenous mouse eIF5A, another feature of this study was the inclusion of a group (Ex6-G6) that was dosed with PEI complexes containing a non-expressing plasmid and an siRNA that efficiently targets mouse eIF5A. This group allowed assessment of the safety of chronic administration of an active eIF5A siRNA.

All animals survived to the scheduled sacrifice date. No clinical signs of toxicity were observed in any of the groups over the course of the 9-week study and the mice in all groups continued to gain weight during the study period. Red and white blood cell counts were measured three and six weeks after the initiation of treatment and were normal for all dosing groups. Serum liver enzyme levels were measured following sacrifice and were within the normal range for all mice. No changes in the macroscopic appearance of the organs were observed in any of the groups. Histopathological analysis of the major organs was conducted by an independent pathologist (and revealed no toxicity attributable to the test article).

Chronic administration of therapeutic doses of SNS-EF1/UU is well tolerated by mice and no adverse effects were observed. In addition, chronic administration of a mouse-specific eIF5A siRNA revealed no toxic effects, indicating that the administration of PEI complexes containing a human eIF5A siRNA should be safe for humans.

#### Example 21

##### Therapeutic Efficacy Studies with Intra-Venous SNS-B29/UU and SNS01 in Multiple Myeloma Tumour-Bearing Mice

SNS01 is as described above. The test article SNS-B29/UU is a preclinical version of SNS01. SNS-B29/UU differs very

little from SNS01, the chief difference being that the components are of research-grade rather than GLP-grade. The purpose of the study reported here was to determine the minimum effective dose of SNS-B29/UU and to confirm that the GLP-grade materials that comprise SNS01 perform as well as the research-grade materials that were used for the preclinical studies. The repeated dose tumour study (Study ID:EX9) was a 5-week study in which the ability of increasingly smaller twice-weekly doses of SNS-B29/UU to inhibit subcutaneous tumour growth in mice was assessed in order to determine the optimal therapeutic dose of SNS01. The treated animals were also assessed for signs of toxicity by monitoring clinical signs, body weight and organ weight.

Study ID	Animal Model	Injection Schedule	Duration of Treatment	Test Article
Ex9	C.B17 (SCID) (female) mice bearing subcutaneous human multiple myeloma tumours (KAS-6/1)	Twice weekly Intra-venous	35 Days	SNS-B29/UU
		Twice weekly Intra-venous	25 Days	SNS01

Test Article	Plasmid DNA	siRNA	Vehicle	Material Grade
SNS01 <sup>a</sup>	pExp5A <sup>c</sup> (eIF5A <sup>K50R</sup> expression driven by B29 B-cell-specific promoter)	eIF5A siRNA <sup>d</sup> (dTdT overhang <sup>c</sup> ) human-specific eIF5A siRNA	In vivo-jetPEI™	GLP-grade materials
SNS-B29/UU <sup>b</sup>	pExp5A <sup>c</sup>	h5A1 (UU overhang <sup>c</sup> ) human-specific eIF5A siRNA	In vivo-jetPEI™	Research-grade materials

<sup>a</sup>SNS01 contains GLP-grade materials and is being developed for the clinic

<sup>b</sup>SNS-B29/UU is a test article used in preclinical research that led to the development of SNS01

<sup>c</sup>the pExp5A plasmid is RNAi-resistant due to the fact that the plasmid contains only the open reading frame of eIF5A while the eIF5A siRNA (h5A1) targets the 3' UTR of eIF5A

<sup>d</sup>the sequence of the eIF5A siRNA and the h5A1 siRNAs are identical except for the presence of a dTdT overhang rather than a UU overhang on the 3' terminal ends of the siRNA; the dTdT overhang does not affect the target selectivity or efficacy of the siRNA but has been proposed to enhance stability

The therapeutic range of SNS-B29/UU was determined in SCID mice bearing subcutaneous human multiple myeloma tumours. Doses of SNS-B29/UU between 0.15 mg/kg and 1.5 mg/kg were tested. The anti-tumoural efficacy of the test article was determined by twice-weekly tumour volume mea-

surements and by excising and weighing tumour tissue following sacrifice. SNS-B29/UU doses of 0.75 mg/kg and 1.5 mg/kg resulted in significant tumour shrinkage indicating that the therapeutic range of SNS-B29/UU lies between 0.75 mg/kg and 1.5 mg/kg. Effective inhibition of growth of subcutaneous tumours was also observed at 0.38 mg/kg SNS-B29/UU although no tumour shrinkage was observed. Some inhibition of tumour growth was even observed at doses as low as 0.15 mg/kg SNS-B29/UU indicating a broad therapeutic range. See FIGS. 26 and 27.

The efficacy of SNS01 made using GLP-grade components was compared to SNS-B29/UU and was found have a com-

parable efficacy in the inhibition of tumour growth. Treatment of tumour-bearing SCID mice with SNS01 and SNS-B29/UU was well tolerated and the mice continued to gain weight throughout the study.

Test Article	Test Article and Vehicle			
	Manufacturer of Plasmid DNA component	Manufacturer of siRNA component	Manufacturer of PEI component	Formation of Test Article
SNS01	VGXI Lot # pExp5A.08L007	Avecia	Polyplus Transfection Inc GLP-Grade	Components were combined with 10% glucose to form nano-complexes; complexes were injected within 2-4 hours
SNS-B29/UU	Qiagen EndoFree Plasmid Mega Kit <0.1 EU/μg DNA	ThermoScientific Dharmacon RNAi Technologies	Polyplus Transfection Inc. Research-Grade	Components were combined with 10% glucose to form nano-complexes; complexes were injected within 2-4 hours

Test Article and Vehicle				
Test Article	Manufacturer of Plasmid DNA component	Manufacturer of siRNA component	Manufacturer of PEI component	Formation of Test Article
Storage Conditions (Stability)	-20° C. (≥1 year)	-20° (≥1 year)	4° (≥1 year)	Room Temperature (≥6 hours)

### Test Systems and Study Design

The female C.B. 17/IcrHsd-Prkdc (SCID) mice were obtained from Harlan (Indianapolis, Ind., USA). Subcutaneous tumours were established by injecting  $10 \times 10^6$  viable KAS-6/1 (human multiple myeloma) cells into the right flank of 5 to 6 week-old mice. Treatment with SNS-B29/UU began when the tumours reached an approximate size of 20 to 40 mm<sup>3</sup> (approximately 4 weeks after tumour cell injection). Treatment with SNS01 began when the tumours reached an approximate size of 130 mm<sup>3</sup> (approximately 6 weeks after tumour cell injection). Mice received the test article twice-weekly via tail vein intra-venous injection.

STUDY ID: EX9						
Group	No. Animals (female)	Test Article	N/P ratio	Dose level (mg/kg)	Injection Volume	Total # injections
Ex9-G1	3	Control <sup>a</sup>	6	1.5 mg/kg	0.1 mL	11
Ex9-G2	4	SNS-B29/UU	6	1.5 mg/kg	0.1 mL	11
Ex9-G3	4	SNS-B29/UU	6	0.75 mg/kg	0.05 mL	11
Ex9-G4	4	SNS-B29/UU	6	0.38 mg/kg	0.025 mL	11
Ex9-G5	3	SNS-B29/UU	6	0.15 mg/kg	0.01 mL	11
Ex9-G9	3	SNS01	6	1.5 mg/kg	0.1 mL	7

<sup>a</sup>PEI complex containing a non-expressing plasmid (same vector background as pExp5A) and a non-targeting siRNA

### Repeated Dose Tumour Study

The repeated dose tumour study was designed to determine the minimum effective therapeutic dose of SNS-B29/UU and to confirm that the GLP-grade SNS01 test article retained the tumour inhibition activity demonstrated by the research-grade test article SNS-B29/UU. A secondary objective was to assess any toxic effects of the treatment by monitoring the treated mice for clinical signs, body weight, and organ weight. Test article therapeutic anti-tumoural activity was monitored by twice-weekly tumour volume measurements using digital calipers. Upon sacrifice the tumours were excised and weighed.

All the mice survived to the scheduled sacrifice date. Control mice that were treated with PEI nanocomplexes containing a non-expressing plasmid and a non-targeting siRNA had an average tumour volume of 284 mm<sup>3</sup> at the time of sacrifice while mice treated with 1.5 mg/kg SNS-B29/UU had an average tumour volume of only 13 mm<sup>3</sup>, a 95% (\*p=0.026) reduction in tumour growth. However, when it was attempted to excise the tumours from mice that had been treated with 1.5 mg/kg SNS-B29/UU, no evidence of a tumour was found in any of the mice. Decreasing the dose of SNS-B29/UU by half to 0.75 mg/kg still resulted in a 91% (\*p=0.03) and 87% (\*p=0.04) decrease in tumour volume and weight, respectively, and in one mouse the tumour had completely disap-

peared. Therefore, the optimum therapeutic dose for twice-weekly injections of SNS-B29/UU appears to be between 0.75 mg/kg and 1.5 mg/kg. Twice-weekly doses of SNS-B29/UU doses as low as 0.15 mg/kg still resulted in a 60% reduction in the final tumour volume indicating that SNS-B29/UU has potent anti-tumoural activity over a wide dose range.

In addition to inhibiting tumour growth, treatment with SNS-B29/UU and SNS01 at 0.75 mg/kg and 1.5 mg/kg resulted in significant reduction in tumour volume indicating that this treatment is capable of inducing tumour regression, likely through the induction of apoptosis in the tumour. The percent change in tumour volume in tumour-bearing mice treated with SNS-B29/UU, at dose levels of 0.75 mg/kg and 1.5 mg/kg, was -244% and -245%, respectively. The tumours of control mice increased in size by more than 2000% during the same time period. Twice-weekly injections of SNS01 also significantly shrunk multiple myeloma tumours. The percent change in tumour volume for mice treated with 1.5 mg/kg SNS01 was -349%, indicating that SNS01 is just as effective as SNS-B29/UU. The use of GLP-grade materials may in fact have increased the biological activity since treatment with SNS01 achieved a 349% decrease in tumour volume following only 25 days of treatment while SNS-B29/UU achieved a 245% reduction in tumour volume following 35 days of treatment. In addition, the tumours treated with SNS01 were quite large (~130 mm<sup>3</sup>) indicating that treatment with SNS01 is effective against well-established tumours.

The treatment was well tolerated by all mice and no clinical signs of toxicity were observed. The mice in all groups continued to gain weight throughout the study. No changes in the macroscopic appearance of the organs was observed at necropsy and no significant changes in the organ weight to body weight ratios occurred.

Therefore SNS01 (and its preclinical version SNS-B29/UU) is well tolerated by SCID mice and is extremely effective in treating subcutaneous human multiple myeloma tumours when delivered by intra-venous injection twice per week. All doses of SNS-B29/UU that were tested were effective at inhibiting tumour growth but the highest dose of 1.5 mg/kg successfully eliminated tumours in all mice receiving treatment.

### Example 22

#### Biodistribution of Plasmid DNA and siRNA Polyethylenimine (JetPEI) Complexes

Green fluorescent protein ("GFP")GFP-expression constructs were used to determine localization of plasmid DNA delivered by PEI complexes. Two promoters were used to drive GFP expression: EF1: ubiquitous promoter (EF1::GFP) or B29: B-cell specific promoter (B29::GFP). PEI complexes containing 20 micrograms of GFP plasmid DNA and 10 micrograms of a fluorescently-labelled (DY547) h5A1

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siRNA were prepared at an N/P ratio of 6. BalB/C mice were injected intra-venously with either 5% glucose or PEI complexes on two consecutive days. Seventy-two hours following the first injection the mice were euthanized and their organs were harvested and analyzed for GFP expression and DY547-siRNA by confocal microscopy.

Bone Marrow:

In most cases there was evidence of DY547-siRNA but no GFP expression. Timing of organ harvest may not coincide with peak expression of GFP; and there may be quenching of GFP signal or GFP may not be expressed. However, GFP and DY547 that colocalized to the same bone marrow cells in some instances was observed. Therefore, this provides evidence that PEI nanoparticles can transfect bone marrow cells in a live animal when given by intra-venous injection

Lung:

In most cases there was evidence of DY547-siRNA but no GFP expression. Timing of organ harvest may not coincide with peak expression of GFP or there may be quenching of GFP signal or GFP may not be expressed.

Spleen:

Evidence of GFP expression (when driven by EF1 promoter) colocalizing in cells also positive for the presence of DY547-siRNA was seen. Expression of GFP was much lower in spleen cells when driven by the B29 promoter. This shows that PEI nanoparticles appear to transfect cells of the spleen.

Kidney:

No GFP or DY547 was observed indicating nanoparticles may not enter kidney.

Liver:

In most cases there was evidence of DY547-siRNA but no GFP expression. This provides evidence that PEI nanoparticles are transfecting cells of the liver.

Heart:

Colocalization of EF1::GFP and DY547-siRNA in tissue of heart was seen, thus indicating that PEI nanoparticles may be transfecting this organ. No GFP was observed with B29 promoter.

## Example 23

Effect of DNA:siRNA Ratio on HA-eIF5A<sup>K50R</sup> Expression

KAS cells were transfected with nanoparticles containing B29-HA-eIF5A<sup>K50R</sup> (plasmid driven by B-cell-specific promoter) and h5A1 siRNA. JET PEI<sup>TM</sup> nanoparticles containing different ratios of pExp5A and h5A1 siRNA were made and incubated for 4 hours at room temperature prior to addition to KAS cells. Four hours after transfection, the nanoparticle-containing media was replaced with fresh media. Twenty-four hours later the cell lysate was harvested and used for western blot analysis with an antibody against HA. The ratio of DNA:siRNA was varied from the standard ratio of

## 52

2:1. The accumulation of HA-eIF5A<sup>K50R</sup> peaked at ratios of 1:0, 3:1, and 2:1. See FIG. 30.

## Example 24

## Effect of DNA:siRNA Ratio on Apoptosis Induced by Nanoparticle Transfection

Nanoparticles containing different ratios of pExp5A and h5A1 siRNA were made and incubated for 4 hours at room temperature prior to addition to KAS cells. Four hours after transfection, the nanoparticle-containing media was replaced with fresh media. Forty-eight hours later the cells were harvested, labelled with Annexin V/PI and analyzed by FACS. The induction of apoptosis was highest in cells transfected with nanoparticles with the standard DNA:siRNA ratio of 2:1. See FIG. 31.

## Example 25

## Administration of PEI Complexes (N/P=6 or 8) Containing eIF5A1K50R Plasmid and eIF5A1 siRNA (siSTABLE or non-siSTABLE) Inhibits Growth of Multiple Myeloma Subcutaneous Tumours and Results in Tumour Shrinkage

SCID mice were injected subcutaneously with KAS cells. Treatment was initiated when palpable tumours were observed. Mice were injected intra-venously 2 times per week with either: (G1) PEI complexes containing 20 mg of pCpG-mcs (empty vector) and 10 mg of control siRNA at N/P=8 (medium dose); (G5) PEI complexes containing 20 mg of the RNAi-resistant plasmid pCpG-eIF5A1k50R and 10 mg of siSTABLE h5A1 siRNA at N/P=8 (medium dose, siSTABLE); (G8) PEI complexes containing 20 mg of the RNAi-resistant plasmid pCpG-eIF5A1k50R and 10 mg of h5A1 siRNA at N/P=6 (medium dose, N/P=6). The data shown is the individual tumour volume for the mice in each group. The final injection was given at day 40 after initiation of treatment. See FIG. 32.

## Example 26

JET PEI<sup>TM</sup> Nanoparticles are being Effectively taken up by Tumour Tissue and that Nanoparticles are Delivering Plasmid and siRNA to the same Cell

Tumour section 48 hours after injection with nanoparticles containing pExp-GFP (GFP under control of B-cell-specific promoter) and DY547-siRNA (fluorescently-labelled siRNA). Co-localized expression of GFP and DY547 is observed in tumour section following confocal microscopy indicating that the nanoparticles are being effectively taken up by tumour tissue and that nanoparticles are delivering plasmid and siRNA to the same cell. See FIG. 33.

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<210> SEQ ID NO 17
<211> LENGTH: 497
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polynucleotide

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<400> SEQUENCE: 17

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acatgtacc ttacgacgtc ccagactacg ctgcagatga tttggacttc gagacaggag 60
atgcaggggc ctcagccacc ttcccattgc agtgctcagc attacgtaag aatggttttg 120
tggtgctcaa gggccggcca tgtaagatcg tcgagatgct tacttcgaag actggcaggc 180
atggccatgc caaggtccat ctggttgcca ttgatatttt tactgggaag aaatatgaag 240
atatctgccc gtcgactcat aacatggatg tcccacaac caaaaggaat gatttccagc 300

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tgattggcat ccaggatggg tacctatccc tgctccagga cagtggggag gtacgagagg 360
accttcgtct gcctgagga gaccttggca aggagattga gcagaagtat gactgtggag 420
aagagatcct gatcacagtg ctgtccgcca tgacagagga ggcagctgtt gcaatcaagg 480
cgatggcaaa ataactg 497

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<210> SEQ ID NO 18
<211> LENGTH: 165
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        polypeptide

```

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<400> SEQUENCE: 18

```

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Met Asp Met Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ala Asp Asp Leu
1          5          10          15
Asp Phe Glu Thr Gly Asp Ala Gly Ala Ser Ala Thr Phe Pro Met Gln
20          25          30
Cys Ser Ala Leu Arg Lys Asn Gly Phe Val Val Leu Lys Gly Arg Pro
35          40          45
Cys Lys Ile Val Glu Met Ser Thr Ser Lys Thr Gly Arg His Gly His
50          55          60
Ala Lys Val His Leu Val Gly Ile Asp Ile Phe Thr Gly Lys Lys Tyr
65          70          75          80
Glu Asp Ile Cys Pro Ser Thr His Asn Met Asp Val Pro Asn Ile Lys
85          90          95
Arg Asn Asp Phe Gln Leu Ile Gly Ile Gln Asp Gly Tyr Leu Ser Leu
100         105         110
Leu Gln Asp Ser Gly Glu Val Arg Glu Asp Leu Arg Leu Pro Glu Gly
115         120         125
Asp Leu Gly Lys Glu Ile Glu Gln Lys Tyr Asp Cys Gly Glu Glu Ile
130         135         140
Leu Ile Thr Val Leu Ser Ala Met Thr Glu Glu Ala Ala Val Ala Ile
145         150         155         160
Lys Ala Met Ala Lys
165

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<210> SEQ ID NO 19
<211> LENGTH: 154
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 19

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Met Ala Asp Asp Leu Asp Phe Glu Thr Gly Asp Ala Gly Ala Ser Ala
1          5          10          15
Thr Phe Pro Met Gln Cys Ser Ala Leu Arg Lys Asn Gly Phe Val Val
20          25          30
Leu Lys Gly Arg Pro Cys Lys Ile Val Glu Met Ser Thr Ser Lys Thr
35          40          45
Gly Lys His Gly His Ala Lys Val His Leu Val Gly Ile Asp Ile Phe
50          55          60
Thr Gly Lys Lys Tyr Glu Asp Ile Cys Pro Ser Thr His Asn Met Asp
65          70          75          80
Val Pro Asn Ile Lys Arg Asn Asp Phe Gln Leu Ile Gly Ile Gln Asp
85          90          95
Gly Tyr Leu Ser Leu Leu Gln Asp Ser Gly Glu Val Arg Glu Asp Leu
100         105         110

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Arg Leu Pro Glu Gly Asp Leu Gly Lys Glu Ile Glu Gln Lys Tyr Asp  
 115 120 125

Cys Gly Glu Glu Ile Leu Ile Thr Val Leu Ser Ala Met Thr Glu Glu  
 130 135 140

Ala Ala Val Ala Ile Lys Ala Met Ala Lys  
 145 150

<210> SEQ ID NO 20

<211> LENGTH: 36

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> SEQUENCE: 20

aattctcgag tcacgataa gcgcccgag acgcgt 36

<210> SEQ ID NO 21

<211> LENGTH: 36

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> SEQUENCE: 21

aattacgcgt ctgcggccgc ttatcgatga ctcgag 36

<210> SEQ ID NO 22

<211> LENGTH: 1030

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
 polynucleotide

<400> SEQUENCE: 22

ggcatgtgaa ctggctgtct tggttttcat ctgtacttca tctgctacct ctgtgacctg 60

aaacatattt ataattccat taagctgtgc atatgataga tttatcatat gtattttcct 120

taaaggattt ttgtaagaac taattgaatt gatacctgta aagtctttat cacactaccc 180

aataaataat aaatctcttt gttcagctct ctgtttctat aaatatgtac aagttttatt 240

gttttttagtg gtagtgattt tattctcttt ctatatatat acacacacat gtgtgcattc 300

ataaatatat acaattttta tgaataaaaa attattagca atcaatattg aaaaccactg 360

atttttgttt atgtgagcaa acagcagatt aaaaggaatt ctcgagtcac cgataagcgg 420

ccgcagacgc gtaattcagt caatatgttc acccaaaaa agctgtttgt taacttgcca 480

acctcattct aaaatgtata tagaagccca aaagacaata acaaaaatat tctttagtaa 540

caaatggga aagaatgttc cactaaatat caagatttag agcaaagcat gagatgtgtg 600

gggatagaca gtgaggctga taaaatagag tagagctcag aaacagacc attgatatat 660

gtaagtgacc tatgaaaaaa atatggcatt ttacaatggg aaaatgatga tctttttctt 720

ttttagaaaa acagggaaat atatttatat gtaaaaaata aaaggggaacc catatgtcat 780

accatacaca caaaaaaaat tccagtgaat tataagtcta aatggagaag gcaaaaacttt 840

aaatctttta gaaaataata tagaagcatg ccatcaagac ttcagtgtag agaaaaattt 900

cttatgactc aaagtcctaa ccacaaagaa aagattgtta attagattgc atgaatatta 960

agacttattt ttaaaattaa aaaaccatta agaaaagtca ggccatagaa tgacagaaaa 1020

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tatttgcaac 1030

<210> SEQ ID NO 23  
 <211> LENGTH: 31  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 23

gaagcggcgc caccacctg ggccaggctg g 31

<210> SEQ ID NO 24  
 <211> LENGTH: 36  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic primer

<400> SEQUENCE: 24

ccacgcgtag aggtgttaa aagtctttag gtaaag 36

<210> SEQ ID NO 25  
 <211> LENGTH: 2282  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 25

ttaattaa ttatctctaa ggcattgtgaa ctggctgtct tggttttcat ctgtacttca 60  
 tctgctacct ctgtgacctg aaacatattt ataattccat taagctgtgc atatgataga 120  
 tttatcatat gtattttcct taaaggattt ttgtaagaac taattgaatt gatacctgta 180  
 aagtctttat cacactacc aataaataat aaatctcttt gttcagctct ctgtttctat 240  
 aaatatgtac aagttttatt gtttttagtg gtagtgattt tattctcttt ctatatatat 300  
 acacacacat gtgtgcatc ataaatatat acaattttta tgaataaaaa attattagca 360  
 atcaatattg aaaaccactg atttttggtt atgtgagcaa acagcagatt aaaaggaatt 420  
 ctcgagtcac cgataagcgc ccgcaccacc ctgggccagg ctgggcccaag ccaggcggcc 480  
 cctgtgtttt cccagctctc tgggctgctg gaggaacca ggttgtttg gcatcagcct 540  
 ctactgagcc ggagccctc ctttctgct gctttgcata gtggcactaa ttccgtctc 600  
 ctacctcac caggaccta ggcagccggg tagatggtgg gaggaggctt cacttctccc 660  
 ccaagcaggg tctccacctg cttgaggctg cctggggtg ggggaggcct tggctttacc 720  
 taaagacttt ttaacacctc tacgcgtaat tcagtcaata tgttcacccc aaaaagctg 780  
 tttgttaact tgccaacctc attctaaaat gtatatagaa gcccaaaaga caataacaaa 840  
 aatattcttg tagaacaaaa tgggaaagaa tgttccacta aatatcaaga tttagagcaa 900  
 agcatgagat gtgtgggat agacagttag gctgataaaa tagagtagag ctacagaaaca 960  
 gaccatttga tatatgtaag tgacctatga aaaaaatag gcattttaca atgggaaat 1020  
 gatgatcttt ttctttttta gaaaaacagg gaaatatatt tatatgtaa aaataaagg 1080  
 gaacccatat gtcataccat acacacaaaa aaattccagt gaattataag tctaaatgga 1140  
 gaaggcaaaa cttaaatct tttagaaaat aatatagaag catgcatca agacttcagt 1200

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gtagagaaaa atttcttatg actcaaagtc ctaaccacaa agaaaagatt gttaattaga 1260
ttgcatgaat attaagactt atttttaaaa ttaaaaaacc attaagaaaa gtcaggccat 1320
agaatgacag aaaatatttg caacacccca gtaaagagaa ttgtaatatg cagattataa 1380
aaagaagtct tacaatcag taaaaaataa aactagacaa aaatttgaac agatgaaaga 1440
gaaactctaa ataatcatta cacatgagaa actcaatctc agaaatcaga gaactatcat 1500
tgcataatac ctaaattaga gaaatattaa aaggctaagt aacatctgtg gcttaattaa 1560
aacaggtagt tgacaattaa acattggcat agtatatctg catagtataa tacaactcac 1620
tataggaggg ccatcatggc caagttgacc agtgctgtcc cagtgtcac agccagggat 1680
gtggctggag ctggtgagtt ctggactgac aggttggggg tctccagaga ttttgtggag 1740
gatgactttg caggtgtggt cagagatgat gtcaccctgt tcatctcagc agtccaggac 1800
caggtggtgc ctgacaacac cctggcttgg gtgtgggtga gaggactgga tgagctgtat 1860
gctgagtgga gtgaggtggt ctccaccaac ttcagggatg ccagtggccc tgccatgaca 1920
gagattggag agcagccctg ggggagagag tttgccctga gagaccagc aggcaactgt 1980
gtgcactttg tggcagagga gcaggactga ggataaccta ggaaacctta aaacctttaa 2040
aagccttata tattcttttt tttcttataa aacttaaac cttagaggct atttaagttg 2100
ctgatttata ttaattttat tgttcaaaca tgagagctta gtacatgaa catgagagct 2160
tagtacatta gccatgagag cttagtacat tagccatgag ggtttagttc attaaacatg 2220
agagcttagt acattaaaca tgagagctta gtacatacta tcaacaggtt gaactgctga 2280
tc 2282

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<210> SEQ ID NO 26
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        primer

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<400> SEQUENCE: 26

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gttatcgata ctagtgcgac cgccaaacc 29

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<210> SEQ ID NO 27
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        primer

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<400> SEQUENCE: 27

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caagcggccg ccataccaca tttgtagagg ttttac 36

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<210> SEQ ID NO 28
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic
        primer

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<400> SEQUENCE: 28

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caccatggca gatgatttg acttc 25

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<210> SEQ ID NO 29
<211> LENGTH: 154

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<212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polypeptide

<400> SEQUENCE: 29

Met Ala Asp Asp Leu Asp Phe Glu Thr Gly Asp Ala Gly Ala Ser Ala  
 1 5 10 15  
 Thr Phe Pro Met Gln Cys Ser Ala Leu Arg Lys Asn Gly Phe Val Val  
 20 25 30  
 Leu Lys Gly Arg Pro Cys Lys Ile Val Glu Met Ser Thr Ser Lys Thr  
 35 40 45  
 Gly Lys His Gly His Ala Lys Val His Leu Val Gly Ile Asp Ile Phe  
 50 55 60  
 Thr Gly Lys Lys Tyr Glu Asp Ile Cys Pro Ser Thr His Asn Met Asp  
 65 70 75 80  
 Val Pro Asn Ile Lys Arg Asn Asp Phe Gln Leu Ile Gly Ile Gln Asp  
 85 90 95  
 Gly Tyr Leu Ser Leu Leu Gln Asp Ser Gly Glu Val Arg Glu Asp Leu  
 100 105 110  
 Arg Leu Pro Glu Gly Asp Leu Gly Lys Glu Ile Glu Gln Lys Tyr Asp  
 115 120 125  
 Cys Gly Glu Glu Ile Leu Ile Thr Val Leu Ser Ala Met Thr Glu Glu  
 130 135 140  
 Ala Ala Val Ala Ile Lys Ala Met Ala Lys  
 145 150

<210> SEQ ID NO 30  
 <211> LENGTH: 3370  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic polynucleotide

<400> SEQUENCE: 30

ttaattaa ttaataaa ttatctctaa ggcattgtgaa ctggctgtct tggttttcat ctgtacttca 60  
 tctgctacct ctgtgacctg aaacatattt ataattccat taagctgtgc atatgataga 120  
 tttatcatat gtattttcct taaaggattt ttgtaagaac taattgaatt gatacctgta 180  
 aagtctttat cacactacc c aataaataat aaatctcttt gttcagctct ctgtttctat 240  
 aaatatgtac aagttttatt gtttttagtg gtagtgattt tattctcttt ctatatatat 300  
 acacacacat gtgtgcatc ataaatatat acaattttta tgaataaaaa attattagca 360  
 atcaatattg aaaaccactg atttttggtt atgtgagcaa acagcagatt aaaaggaatt 420  
 ctcgagtcac cgatactagt gcgaccgcca aaccttagcg gccagctga caaaagcctg 480  
 ccctcccca gggctcccgg agagctggtg cctcccctgg gtcccaattt gcatggcagg 540  
 aaggggcctg gtgaggaaga ggcggggagg ggacaggctg cagecgggtgc agttacacgt 600  
 tttctccaa ggagcctcgg acgttgtaa gcttctgect tctccctcct gtgagtttgg 660  
 taagtactg actgtctatg cctgggaaag ggtgggcagg agatggggca gtgcaggaaa 720  
 agtggcacta tgaaccctgc agccctagga atgcatctag acaattgtac taaccttctt 780  
 ctctttcctc tcctgacagg ttggtgtaca gtagcttoca ccatggcaga tgatttgac 840  
 ttcgagacag gagatgcagg ggccctcagc accttcccaa tgcagtgtc agcattacgt 900  
 aagaatggtt ttgtggtgct caagggccgg ccatgtaaga tcgtcgagat gtctacttctg 960



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aagactggca	ggcatggcca	tgccaaggtc	catctggttg	gtattgatat	ttttactggg	1020
aagaaatatg	aagatatctg	cccgtcgact	cataacatgg	atgtccccaa	catcaaaagg	1080
aatgatttcc	agctgattgg	catccaggat	gggtacctat	ccctgctcca	ggacagtggg	1140
gaggtacgag	aggaccttcg	tctgcctgag	ggagaccttg	gcaaggagat	tgagcagaag	1200
tatgactgtg	gagaagagat	cctgatcaca	gtgctgtccg	ccatgacaga	ggaggcagct	1260
gttgcaatca	aggcgatggc	aaaataactg	gctagctggc	cagacatgat	aagatacatt	1320
gatgagtttg	gacaaaccac	aactagaatg	cagtgaaaaa	aatgctttat	ttgtgaaatt	1380
tgtgatgcta	ttgctttatt	tgtaaccatt	ataagctgca	ataaacaagt	taacaacaac	1440
aattgcattc	atthttatgtt	tcaggttcag	ggggaggtgt	gggaggtttt	ttaaagcaag	1500
taaaaacctct	acaaatgtgg	tatggcggcc	gcaccacct	gggccaggct	gggccaagcc	1560
aggcggcccc	tgtgttttcc	ccagtctctg	ggctgctgga	gggaaccagg	ttgttttggc	1620
atcagcctct	actgagccgg	agcccttct	ttctgctgc	tttgcatagt	ggcactaatt	1680
ccgtcctcct	acctccacca	gggacctagg	cagccgggta	gatggtggga	ggaggcttca	1740
cttctcccc	aagcagggtc	tccacctgct	tgaggctgcc	ctgggttggg	ggaggccttg	1800
gctttacctta	aagacttttt	aacacctcta	cgcgtaattc	agtcaatatg	ttcaccctca	1860
aaaagctggt	tgtaacttg	ccaacctcat	tctaaaatgt	atatagaagc	ccaaaagaca	1920
ataacaaaaa	tattcttgta	gaacaaaatg	ggaaagaatg	ttccactaaa	tatcaagatt	1980
tagagcaaag	catgagatgt	gtggggatag	acagtgaggc	tgataaaata	gagtagagct	2040
cagaaacaga	cccattgata	tatgtaagtg	acctatgaaa	aaaatatggc	atthttacaat	2100
gggaaaatga	tgatcttttt	ctthttttaga	aaaacaggga	aatatattta	tatgtaaaaa	2160
ataaaaaggga	acctatatgt	cataccatac	acacaaaaaa	attccagtga	attataagtc	2220
taaatggaga	aggcaaaact	ttaaactctt	tagaaaataa	tatagaagca	tgccatcaag	2280
acttcagtgt	agagaaaaat	ttcttatgac	tcaaagtcct	aaccacaaag	aaaagattgt	2340
taattagatt	gcatgaatat	taagacttat	thttaaaatt	aaaaaacct	taagaaaagt	2400
caggccatag	aatgacagaa	aatatthtga	acacccctag	aaagagaatt	gtaatatgca	2460
gattataaaa	agaagtctta	caaatcagta	aaaaataaaa	ctagacaaaa	atthtgaacag	2520
atgaaagaga	aactctaaat	aatcattaca	catgagaaac	tcaatctcag	aaatcagaga	2580
actatcattg	catatacact	aaattagaga	aatatthaaa	ggctaagtaa	catctgtggc	2640
ttaattaaaa	caggtagttg	acaattaaac	atthgcatag	tatatctgca	tagtataata	2700
caactcacta	taggagggcc	atcatggcca	agthgaccag	tgctgtccca	gtgctcacag	2760
ccagggatgt	ggctggagct	gttgagttct	ggactgacag	gtthgggttc	tccagagatt	2820
thtggaggga	tgactthtga	ggtgtggtca	gagatgatgt	cacctgttc	atctcagcag	2880
tccaggacca	ggtggtgcct	gacaacacct	tggcttgggt	gtgggtgaga	ggactggatg	2940
agctgtatgc	tgagtggagt	gagggtgtct	ccaccaactt	cagggatgcc	agtggccctg	3000
ccatgacaga	gattggagag	cagccctggg	ggagagagtt	tgccctgaga	gaccagcag	3060
gcaactgtgt	gcactthtgt	gcagaggagc	aggactgagg	ataacctagg	aaacctthaa	3120
acctthaaaa	gccttatata	thctthtttt	tctthataaaa	ctthaaaacct	tagaggctat	3180
ttaagthtgc	gatttatatt	aatthttattg	thcaaacatg	agagcttagt	acatgaaaca	3240
tgagagctta	gtacattagc	catgagagct	tagtacatta	gccatgaggg	thtagthcat	3300
taaacatgag	agcttagtac	atthaacatg	agagcttagt	acatactatc	aacaggttga	3360

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actgctgac 3370

<210> SEQ ID NO 31  
 <211> LENGTH: 21  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Combined DNA/RNA Molecule:  
 Synthetic oligonucleotide  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> SEQUENCE: 31

gcuggacucc uccuacacat t 21

<210> SEQ ID NO 32  
 <211> LENGTH: 21  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Combined DNA/RNA Molecule:  
 Synthetic oligonucleotide  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
 oligonucleotide

<400> SEQUENCE: 32

uguguaggag gaguccagct t 21

<210> SEQ ID NO 33  
 <211> LENGTH: 537  
 <212> TYPE: DNA  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
 polynucleotide

<400> SEQUENCE: 33

ataggagac ccaagctgga gaccatgtac ccatacgacg tcccagacta cgctggaagc 60  
 ttaatggcag atgatttga ctctgagaca ggagatgcag gggcctcagc caccttccca 120  
 atgcagtgt cagcattacg taagaatggt tttgtggtgc tcaagggccg gccatgtaag 180  
 atcgtcgaga tgtctacttc gaagactggc aggcatggcc atgccaaggt ccatctggtt 240  
 ggtattgata ttttactgg gaagaaatat gaagatatct gcccgctgac tcataacatg 300  
 gatgtcccca acatcaaaag gaatgatttc cagctgattg gcatccagga tgggtaccta 360  
 tcctgctcc aggacagtgg ggaggtacga gaggaccttc gtctgctga gggagacctt 420  
 ggcaaggaga ttgagcagaa gtatgactgt ggagaagaga tcctgatcac agtgctgtcc 480  
 gccatgacag aggaggcagc tgttgcaatc aaggccatgg caaaataact ggaattc 537

<210> SEQ ID NO 34  
 <211> LENGTH: 167  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
 polypeptide

<400> SEQUENCE: 34

Met Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Gly Ser Leu Met Ala Asp  
 1 5 10 15

Asp Leu Asp Phe Glu Thr Gly Asp Ala Gly Ala Ser Ala Thr Phe Pro  
 20 25 30

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Met Gln Cys Ser Ala Leu Arg Lys Asn Gly Phe Val Val Leu Lys Gly  
           35                                  40                                  45

Arg Pro Cys Lys Ile Val Glu Met Ser Thr Ser Lys Thr Gly Arg His  
       50                                  55                                  60

Gly His Ala Lys Val His Leu Val Gly Ile Asp Ile Phe Thr Gly Lys  
   65                                  70                                  75                                  80

Lys Tyr Glu Asp Ile Cys Pro Ser Thr His Asn Met Asp Val Pro Asn  
                                   85                                  90                                  95

Ile Lys Arg Asn Asp Phe Gln Leu Ile Gly Ile Gln Asp Gly Tyr Leu  
                                   100                                  105                                  110

Ser Leu Leu Gln Asp Ser Gly Glu Val Arg Glu Asp Leu Arg Leu Pro  
                                   115                                  120                                  125

Glu Gly Asp Leu Gly Lys Glu Ile Glu Gln Lys Tyr Asp Cys Gly Glu  
       130                                  135                                  140

Glu Ile Leu Ile Thr Val Leu Ser Ala Met Thr Glu Glu Ala Ala Val  
   145                                  150                                  155                                  160

Ala Ile Lys Ala Met Ala Lys  
                                   165

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The invention claimed is:

1. A composition comprising a complex of an eIF5A1 siRNA targeted against the 3' end of eIF5A1, and an expression vector comprising a polynucleotide encoding a mutant eIF5A1 that contains a K50R mutation in the eIF5A1 amino acid sequence set forth in SEQ ID NO: 18, wherein the siRNA and the expression vector are complexed to polyethylenimine.

2. The composition of claim 1 wherein the siRNA has the sequence of SEQ ID NO: 31 and the complement thereof (SEQ ID NO: 32).

3. The composition of claim 2 comprising a tissue specific promoter.

4. The composition of claim 2 wherein the expression vector comprises a pCpG plasmid.

5. The composition of claim 1 wherein the eIF5A1 siRNA and the expression vector comprising the mutant eIF5A1 polynucleotide are independently complexed to polyethylenimine.

6. The composition of claim 1 wherein the eIF5A1 siRNA and the expression vector comprising the mutant eIF5A1 polynucleotide are together complexed to polyethylenimine.

7. The composition of claim 1 wherein the eIF5A1 siRNA targeted against the 3' end of eIF5A1 and an expression vector comprising a polynucleotide encoding a mutant eIF5A1 present in an amount effective to induce apoptosis in multiple myeloma cells in a human subject suffering from multiple myeloma.

\* \* \* \* \*